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THE ANNALS OF APPLIED BIOLOGY

THE OFFICIAL ORGAN OF THE ASSOCIATION
OF ECONOMIC BIOLOGISTS

EDITED BY
PROFESSOR MAXWELL LEFROY, Imperial College of Science and Technology, London
AND
PROFESSOR B. T. P. BARKER, National Fruit and Cider Institute, Bristol
DR S. E. CHANDLER, Imperial Institute, London
F. J. CHITTENDEN, Royal Horticultural Society's Gardens, Wisley
PROFESSOR F. W. GAMBLE, The University, Birmingham
E. E. GREEN, late Government Entomologist, Ceylon
PROFESSOR PERCY GROOM, Imperial College of Science and Technology, London
DR A. D. IMMS, The University, Manchester
PROFESSOR R. NEWSTEAD, The University, Liverpool
PROFESSOR J. H. PRIESTLEY, The University, Leeds

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VOLUME II MAY, 1915 No. 1

A BACTERIAL DISEASE OF THE MANGO.

BACILLUS MANGIFERAE N.SP.

BY ETHEL M. DOIDGE, M.A., F.L.S.,

Mycologist, Division of Botany, Pretoria.

(*Thesis approved for the Degree of Doctor of Science in the University of the Cape of Good Hope.*)

(With Plates I—XIV and 3 Text-figures.)

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FOR some years past, fruit growers in certain districts of this country have been troubled by a disease of mangoes which is causing considerable havoc in their orchards and threatens to seriously affect the export trade.

All parts of the tree are attacked, the affected areas becoming discoloured, while in fruit and stem deep longitudinal cracks also occur; the disease has been described by farmers as resembling in appearance the anthracnose of the grape vine caused by *Gloeosporium ampelophagum* Sacc., and its external effects are not dissimilar to those caused by that fungus. A large percentage of the fruit falls to the ground whilst yet immature, and the mangoes which remain on the trees are rendered unsightly and unfit for the market.

The disease was first reported in December, 1909, as occurring at Barberton in the Transvaal, and as being unchecked in its progress by repeated applications of Bordeaux mixture. It was soon found that most of the mango-growing districts of the Union were similarly affected and it was considered advisable to make a more detailed study of the disease and the organism causing it, with a view to formulating, if possible, some method by which its progress might be checked. This purpose has not yet been fully carried out as no effective remedy has been found, but the work has progressed to a point where it seems advisable that the results which have been obtained should be placed on record.

Literature.

There is no description in available literature of a disease of mangoes in any way resembling the one under discussion. General literature on the mango is very scanty and consists for the most part of pamphlets describing varieties and methods of propagation. In certain of these, however, reference is made to the diseases to which the mango is subject, and a short review of such references may not be out of place here.

Three diseases are reported from Hawaii (7), a blight caused by *Colletotrichum* sp.; mango scab, and sooty mould. In Hawaii and Cuba the bloom blight due to *Gloeosporium mangiferae* is common, attacking the opening blossoms and causing them to turn black, dry up and fall (3 and 6). This disease appears to be common in most districts where the mango is cultivated and has been reported in this country from Natal.

There is no reference to any fungous or bacterial trouble in a pamphlet entitled *The Mango, its Culture and Varieties*, by C. Marshall Woodrow (12), although remedies are suggested against certain insect pests in a paragraph on the "Enemies of the Mango Tree."

"The mango in Porto Rico seems almost entirely free from disease, or the attacks of insects. On the north side of the island the skin of the fruit is frequently disfigured by black spots, probably a fungus. Though in no way injuring the eating quality of the fruit, these detract from its appearance....In drier localities this discolouration was not observed" (4).

"Some of the varieties of mangoes cultivated in Trinidad are liable to a disease which makes the interior of the fruit assume a dark, jelly-like consistency, and the pulp immediately surrounding the seed becomes completely sour and uneatable. This does not arise from being over-ripe, as the same state of things may be seen in a half-ripe mango if cut. Many of the trees in the Botanic Gardens are affected, and hitherto we have been unable to ascertain the immediate cause."

"It is curious to note, however, that in some cases individual trees only are affected, while others growing near by produce perfectly sound fruit. It is certainly more prevalent with the better class of fruit than with the commoner kinds, but it is to be seen in all more or less. Again, some of the fruit of a tree may be perfectly sound, while another portion just as carefully handled will be utterly useless" (13).

There is no mention of any disease in a paper on "The Mango in South California" (9).

Of the six diseases mentioned above, four are due to the attacks of fungi; the remaining two, of which the cause has not been determined, differ essentially in their effects from the one to be described.

Geographical distribution.

Our attention was first drawn to this disease by Messrs Winter Bros. of Barberton, who sent specimens of mangoes from their trees for examination. They stated that it made its first appearance there after the great hail storm in October 1906, which damaged the trees considerably; the marks of the hail are still visible on the trunks and larger branches. A tree standing in a corner of the orchard was the first to become infected, and from this point the infection rapidly spread with the prevailing winds until every tree was diseased. In 1908, in spite of repeated spraying with Bordeaux mixture, they obtained not a single fruit from 60 trees. Almost all the fruit in the town was similarly affected, but at the farms, some distance away, the fruit was then perfectly sound. Every season up to the present (1913–14) the disease has been steadily gaining ground, each year spreading to some orchard which was previously free from infection, and this in spite of the fact that there have been four very dry seasons in succession, a condition which—as will be shown later—is highly unfavourable to the spread of the disease.

In 1910, a sample of infected fruit was received from Warmbaths. On making an inspection of the orchard in which the fruit was grown, it was found that the infection had started at a corner of the orchard where some young trees had been planted. These young trees were badly infected, and, as at Barberton, the infection had spread with the prevailing winds until a large proportion of the trees was affected. The trees which were apparently the original source of infection had been purchased in Natal, and it seemed probable that in this case the disease had been introduced from that province.

The writer visited Natal in March 1910, with a view to discovering whether this was the case, and found that it was prevalent throughout the mango-growing districts of Natal. It was actually observed on the leaves and stems of trees at Hillarys, Malvern and Durban. During a later visit in December 1911, it was seen on the fruit in all these localities.

In 1910, Mr R. A. Davis, the Government Horticulturist, reported that he had examined trees in Swaziland and found them quite healthy. These trees had all been imported from Natal some 14 years previously, and this would seem to point to the fact that the disease was not known in Natal at that time. This idea was confirmed by a statement made by Dr Medley Wood, who was, until recently, Director of the Durban Botanic Gardens, and who informed me that whereas some years back he sold mangoes from the trees in the gardens up to the value of £30, recently he had scarcely obtained sufficient for his own use.

In August, 1911, an inspection of the mango trees at the Tzaneen and Westphalia Estates in the Zoutpansberg District showed them to be quite free from this trouble.

This disease was noted on some mango fruits from Lourenço Marques in November, 1910; but in Portuguese East Africa would appear to be confined to the neighbourhood of Delagoa Bay; for in a letter dated 14th November, 1913, Mr Pole Evans writes: "When I visited Portuguese East Africa in September last, I saw some of the largest mango trees that I have ever seen at Quilimane and Inhambane. The trees were then in flower. I examined carefully for any disease that might be present, and saw no evidence of the bacterial disease. The inhabitants told me that the trees bore well and that the fruit was clean."

In the absence of any reference in available literature to any disease at all resembling the one under discussion, an attempt was made by various means to discover whether it occurred outside South Africa or not.

The symptoms accompanying the disease, which are very characteristic, were described to Dr Erwin F. Smith of Washington, and he stated that he was not aware of the existence of such a disease in America.

With reference to the possible occurrence of this trouble in India, Dr E. J. Butler, Director of the Imperial Agricultural Research Institute, Pusa, in a letter dated the 29th February, 1912, writes as follows:

"Last year for the first time a supposed bacterial disease of mango fruits was reported from the Bengal Agricultural College, Sabour, Bhagalpur. The Indian Assistant Professor of Mycology who examined the disease could find no fungus in the rotted spots on the fruit. Bacteria were naturally present, and he attributed the damage, which was considerable, to this cause. Some fruits were sent to my Laboratory and on examination I found the common *Gloeosporium Mangae* Noack (probably *G. Raciborski*, common on the leaves) together with a little of a Hendersonia which we have previously found associated with a mango fruit rot. I found no evidence to support the idea that the disease was bacterial."

At the suggestion of Mr R. A. Davis, we communicated with Mr Cousins, Director of Agriculture in Jamaica, who has devoted considerable time and study both to the cultivation of the mango and to the diseases to which it is subject. Mr Cousins wrote in reply:

"This disease is quite unknown in Jamaica. The mango sometimes suffers from the attack of thrips on the young foliage but otherwise is almost immune from disease and pests in Jamaica."

The majority of the trees in Natal were grown in the first instance from seed imported from Mauritius. Mr Bijoux, the Assistant Director of the Botanic Gardens, Mauritius, was visiting Durban in December, 1911. He stated that when he left the island the trees were perfectly healthy and laden with fruit. He knew nothing of the occurrence of such a disease. This information was obtained through the courtesy of Dr Medley Wood.

To summarise: so far as can be ascertained, there is no evidence of the occurrence of this disease outside South Africa. In all the mango-growing districts of the Union with the exception of the Zoutpansberg, the disease has caused considerable loss. Swaziland is also free from infection. In Portuguese East Africa cases of infection have only been reported from the neighbourhood of Delagoa Bay.

Symptoms of the disease.

Although the effects of the disease on the fruit are the most conspicuous, and of the greatest importance economically, numerous infections also occur on stems and leaves. The latter do not greatly affect the general health of the tree, but they serve to carry over the infection from one season to the next.

The first signs of infection noticeable on the leaves are a number of small, angular, water-soaked areas bounded by some of the veins of the leaf. These spots do not increase much in size and rarely exceed 2—3 mm. in diameter; but if they are very numerous they coalesce and larger spots are formed. The infected areas soon begin to discolour and become dark brown, the surface is somewhat raised and shining and frequently there is a slight exudation of gum (Plate II). In very old leaves these discoloured spots become white and dry, and crack away. If infection takes place in the petioles—as is often the case—longitudinal cracks result which attain a length of 1 cm. or less.

Diseased spots are also found on all parts of the stem, although it is evident that the majority of the infections take place in young and rapidly growing tissues; they are also common in the scars from which leaves have newly fallen. The first appearance of disease in the stem is a discolouration of the tissues. This is accompanied by gummosis and the formation of deep longitudinal cracks. The discolouration is not merely superficial but penetrates some distance into the stem (Plate III).

The disease is especially evident on the peduncles and pedicels; frequently, by the time the fruit is half grown, the whole inflorescence is affected. The stalks to which the mangoes are attached become black and dead, and consequently the fruit all falls to the ground (Plate VI).

As has been mentioned above, although the spots on the leaves and stems are often very numerous, they do not noticeably affect the general health of the tree. Their chief importance is as a source of infection for the fruit which is seriously injured by the disease. Large numbers of mangoes fall to the ground, and the small percentage which remains on the trees is so disfigured as to be practically useless (Plate IV). The mangoes on the windward side of the tree suffer most. The diseased spots first appear on the most exposed side of the fruit or else at the spot where two mangoes on the same bunch are in contact and where a drop of water would lodge after rain. The first sign of infection is a small water-soaked area round the white spot which indicates the presence of a stoma, or near a slight wound; this spreads considerably and then begins to discolour. Cracking takes place in several directions and the surface of the diseased area becomes very much roughened. If infection takes place during a period of rapid growth, deep longitudinal cracks are formed, running almost the whole length of the fruit in bad cases (Plate V).

The discoloured spots vary much in size; they are 1 mm. to 15 mm. in diameter and are irregular in shape, and the discolouration penetrates to a depth of 8—15 mm.

Especially noticeable in the case of infections near the point of attachment of the fruit is the exudation of gum which runs over the surface of the mango. This substance is highly infectious and diseased spots are developed wherever it touches the surface of the fruit.

When a mango has once become diseased the slightest air movement detaches it from the tree, and the ground becomes strewn with decaying fruit.

Hosts and Varieties affected.

The disease has been observed on all the varieties of mango commonly grown in this country, but all are not equally susceptible.

The variety known locally as the Peach Mango or Figette is the most resistant. This is a very fine-looking mango and keeps well, but is said to be of inferior flavour.

The most susceptible is the "Baissac" variety, known locally as the "Long Green"; the other three varieties which are commonly grown, namely "Maison Rouge," "Corde" and "Dauphine," are almost as badly affected. These are known locally as the "Long Red," or "Red Kidney," the "Common Yellow" and the "Round Green," respectively.

In view of the fact that so far as is known at present this disease is confined to South Africa, search was made among nearly related indigenous plants for a source of infection. In particular, the "Maroola" tree (*Sclerocarya caffra*) was examined, as it is very common in districts where the mango is grown. The only specimen of this plant which showed any spots on the leaves proved to be infected with *Cercospora* sp.; and I have been unable to produce with the bacillus causing the mango disease, any infections on the leaves or fruit of the tree.

Spraying experiments.

A more detailed study of the progress of the disease was made during the season 1911—1912 in connection with spraying experiments which were being carried out at Barberton in an orchard kindly placed at our disposal by Messrs Winter Brothers. The season was an exceptionally dry one, and consequently unfavourable to the spread of the disease. The following table compiled from statistics furnished by the Meteorological Department shows that the rainfall has been much lower than in the previous seasons. The temperature has been exceptionally high.

Comparative Table. Rainfall 1907—1912.

	September		October		November		December		January		Total	
Season	No. of ins.	No. of days	No. of ins.	No. of days	No. of ins.	No. of days	No. of ins.	No. of days	No. of ins.	No. of days	No. of ins.	No. of days
1907–8	·14	2	2·18	9	4·14	15	4·15	10	3·62	9	14·59	45
1908–9	2·38	3	2·42	12	3·76	12	3·78	8	14·99	17	27·33	52
1909–10	·63	4	1·78	7	3·70	13	6·74	12	5·51	15	18·36	51
1910–11	1·14	5	4·13	14	3·88	13	5·41	13	7·95	10	22·51	55
1911–12	·07	1	4·63	13	2·35	10	2·61	12	1·74	9	11·40	45

The orchard in which the spraying experiments were carried out is situated on a slope to the east of the town, and contains 28 trees arranged in four rows of seven trees each. The trees are ten or twelve years old and comparatively small—about 12 feet high and 35 feet in circumference—so that the spraying could be thoroughly done.

There is a range of hills to the south of the orchard and the prevailing winds are from the south-east. The disease had started in the south-east corner of the orchard, and from there had spread right through. Before the experiments were commenced the trees were all diseased: row (1) being bad right through while in the other rows trees (6) and (7) were the worst.

Plan of Winter Bros. Orchard, Barberton.

East 200 ft.

						*	* ↙	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	Row 1.
North	(1)	(2)	(3)	(4)	(5)	(6)	(7)	Row 2.
150 ft.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	Row 3.
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	Row 4.

Four Rows of Mango Trees, 18 feet apart.
The arrow indicates the direction of the prevailing winds.
* These trees were the first to contract the disease.

Rows 1 and 3 were sprayed with an iron sulphide solution, using the following formula:

Quicklime	4 lbs.
Flowers of sulphur ..	4 ,,
Iron sulphate	1½ ,,
Water	25 gallons

and following the directions given in the circular published by this Division.

Rows 2 and 4 were sprayed with Bordeaux mixture, using a 2–2–50 formula for the first spraying and a 4–4–50 in subsequent applications.

In each row tree No. (3) was left unsprayed as a control.

The experiment was carried out by Mr P. A. van der Bijl, M.A., of this Division, and he was assisted in the earlier part of the work by Mr H. F. Benger. During the seasons 1912—1913 and 1913—1914, the spraying was also done by Mr van der Bijl, and it is from his reports that this account of the spraying experiments is compiled.

The following table gives the result of the experiment in connection with weather conditions, etc. The slight discrepancy between this and the previous table is due to the fact that the first gives the rainfall in the whole district, the second in Barberton only.

Season 1911—12.

Week ending	Rainfall	Temperature		Remarks on Weather	Treatment	Development of Fruit	Progress of Disease
		Max.	Min.				
26. 8. 11	None			No rain up to 30. 9. 11	1st spraying	Trees in flower	Old leaves badly diseased
7. 10. 11	2·66 in.	74°F.	52°F.	First heavy rain of season			
14. 10. 11	None						
21. 10. 11	·01 in.				2nd spraying	Fruit about the size of walnuts	No sign of disease on fruit
28. 10. 11	·61 in.	70°F.	58°F.	Three weeks since 1st rain		Fruit 1½″ to 2″ long	Diseased spots first noticed on fruit
4. 11. 12	·87 in.	74°F.	52°F.				
11. 11. 11	·08 in.	78°F.	54°F.		3rd spraying		About 5 % of fruit diseased
18. 11. 11	·46 in.	86°F.	54°F.				
25. 11. 11	1·29 in.						
2. 12. 11	2·06 in.	82°F.	64°F.		4th spraying	Fruit 3–4 ins. long	Disease spreading. About 7 % disease
9. 12. 11	·58 in.						
16. 12. 11	·45 in.						
23. 12. 11	·6 in.	98°F.	64°F.				
30. 12. 11	·5 in.	90°F.	58°F.				
6. 1. 12	·86 in.	100°F.	62°F.				
13. 1. 12		88°F.	54°F.				
20. 1. 12	·11 in.	92°F.	62°F.			Almost full grown	Fruit on all trees diseased 40–70 % affected

Diseased spots were first observed on the fruit about three weeks after the first rain of the season.

The final estimate of the percentage of diseased fruit refers only to the mangoes still on the trees; large numbers had fallen to the ground and rotted.

The result of the experiment was far from encouraging. On the 20th January an estimate of the number of mangoes still on the trees showed 30·7 % free from disease on the trees sprayed with iron sulphide solution; 54 % on those sprayed with Bordeaux mixture and 67 % on the control trees.

A second spraying experiment was carried out during the season 1912—13, but unfortunately weather conditions were again unfavourable.

The spraying was done in the orchard described above, trees Nos. 3, 5 and 7 in each row being left as controls and the remainder being sprayed with Hycol in the proportion of half a pint to 40 gallons of water (1 in 600). The trees were sprayed four times, and at the time for the first and second sprayings the ground was disinfected with Hycol in the proportion of half a pint of Hycol to 20 gallons of water (1 in 300).

The season was an exceptionally dry one, the fruit developing slowly, and some trees failing to set any fruit at all.

Owing to the drought, the disease spread very slowly early in the season; the first infections on the fruit being observed early in December. The trees were sprayed for the fourth time on the 4th December and on this occasion it was computed that ·45 % of the fruit on the sprayed trees was infected and 1·2 % of that on the unsprayed.

In January the experiment was discontinued owing to the fact that the fruit had been severely cut up by a hailstorm on the 20th of December. On the 7th of January it was calculated that the unsprayed trees had dropped 36·1 % of their fruit and the sprayed trees 38·8 % since the previous examination. Of the fruit which remained on the tree there was 16·7 % of the fruit on the unsprayed trees diseased as compared with 7·3 % on the sprayed trees. The latter seemed to have made more growth than the former, and the Hycol had not damaged the fruit or foliage in any way. The trees which were sprayed with iron sulphide solution during the previous season had received a severe check.

The results of this experiment as far as they went were more encouraging than those of the previous one, but owing to their having been brought to such a premature end they were not sufficiently conclusive, and a further test was necessary. A table is appended showing the rainfall, temperature, etc., during the season.

The spraying with Hycol was continued during the season 1913—14. The first spraying was done on the 29th August, when the trees and the soil underneath them were thoroughly drenched with Hycol 1·600. The trees were then in flower.

Season 1912—13.

Week ending	Rainfall	Temperature		Remarks on Weather	Treatment	Development of Fruit	Progress of Disease
		Max.	Min.				
11. 9. 12	Nil.	—	—	Sun and wind	Sprayed with Hycol	Blossoming	No new infections yet evident
18. 9. 12	,,	—	—	Hot sun		Blossoms and fruit developing slowly on account of drought	
25. 9. 12	,,	—	—	Sun and wind			
2. 10. 12	,,	83°F.	45°F.	Sun and cloud	Sprayed with Hycol		
9. 10. 12	,,	92°F.	45°F.	Sun and cloud			
16. 10. 12	,,	99°F.	50°F.	Sun		Very backward through drought	
23. 10. 12	Drops	107°F.	60°F.	Sun and cloud			
30. 10. 12	·49 in.	90°F.	52°F.	Sun and showers	Sprayed with Hycol		
6. 11. 12	Nil.	106°F.	62°F.	Sun			
13. 11. 12	,,	108°F.	59°F.	Sun and cloud		Fruit small through drought	
20. 11. 12	2·18 in.	97°F.	48°F.	Sun and rain			
27. 11. 12	Nil.	110°F.	55°F.	Sun			Disease now spreading rapidly
4. 12. 12	·05 in.	103°F.	66°F.	Sun and cloud	Sprayed with Hycol		
11. 12. 12	2·51 in.	105°F.	65°F.	Sun and storm		Much damaged by hail	Disease now spreading fast
18. 12. 12	·3 in.	95°F.	62°F.	Sun and cloud			
25. 12. 12	1·5 in.	96°F.	57°F.	Sun and heavy hail storm	Experiment discontinued	Nearly all fruit cut off by hail and the remainder bearing hail marks	

The second spraying was done with Hycol 1 : 300 on the 6th October, after the fruit had set and was 2—3 cm. in length. On the third application of the spray on the 15th of November, the fruit had attained a length of 4—5 cm. and the disease had begun to appear both on the sprayed trees and the controls.

On the 26th January, Mr van der Bijl paid his final visit to Barberton in connection with the spraying experiments. The fruit on both sprayed trees and controls had nearly all fallen, and most of those which remained were diseased. It is therefore evident that Hycol is useless in preventing the spread of the disease.

This was again a dry season. About $2\frac{1}{2}$ inches rain fell between the first and second sprayings, and about 2 inches accompanied by hail between the second and third. The fruit was slightly injured by the hailstones.

Mr Winter reported that the disease has this season spread through the valley, and that he has noticed diseased fruit on his farm at some distance from the village.

From the account of spraying experiments given above, it is evident that the sprays used up to the present are useless in combating this disease; and that iron sulphide is worse than useless as it injures the foliage and thus promotes the spread of infection. The mango being an evergreen tree it is impossible to check the disease by pruning as in the case of "fire blight" and other diseases of deciduous trees.

The risk of infection can be slightly reduced by gathering and burning all diseased fruit and leaves and by keeping the soil under trees damp with some germicide which will prevent the dust from blowing about in the wind and carrying with it the infective bacteria; but no remedial measures used up to the present can be recommended as being really effectual.

The cause of the disease.

A large number of diseased plants have been examined, and in every case the tissues of the discoloured areas in the leaves, stem and fruit were crowded with bacteria.

An organism was isolated from a diseased fruit from Barberton in November, 1909; as it occurred in the host it was a short rod with rounded ends about $1{\cdot}5 \times {\cdot}6\,\mu$. A pure culture was obtained without any difficulty. The same organism has since been isolated dozens of times from fruit and leaves of trees growing in Warmbaths, Barberton and several localities in Natal; in every instance an almost pure culture was obtained at once, except in the case of very old infections on the fruit where a number of yeasts and bacilli have established themselves.

The method used in isolating the organism was as follows: A fairly young infection on the fruit was selected, the surface seared with a hot knife, then a portion of the underlying tissues was cut out with a hot sterile knife and dropped into a tube of sterile distilled water; from this a series of plates was poured.

A different method was adopted with the leaves because the organism diffuses very slowly into water from the leaf tissues. A leaf was washed in 1 : 1000 mercuric chloride, then in several lots of distilled water. A glass pestle and mortar similarly treated, and then an infected part of the leaf ground up in it with some sterile sand and a little distilled water. A drop of the liquid was put into a tube of medium and plates poured.

After the organism had been repeatedly isolated in the way described above, attempts were made to infect a number of young mango trees. The inoculation work has been rendered difficult by the entire absence of any greenhouse accommodation. The trees do not thrive in the open in Pretoria and though they have flowered for two seasons they have not developed any fruit.

It has already been shown that the majority of the natural infections take place in vigorously growing tissues during a spell of wet weather. There have been three very dry seasons since the inoculation work was commenced (1911—12), and it is almost impossible to obtain infections in the open in dry weather.

The few successful inoculations described below, were obtained during a short spell of rain and cloud. There have been a proportionally large number of failures, but in no case have any of the control plants become infected.

This phase of the work will be continued as soon as suitable greenhouse accommodation is available.

Direct infection experiments.

1. A small portion of the gummy substance exuding from an infected spot on a mango fruit was inoculated by needle pricks into the stem and petioles of a young mango tree. After about four weeks there were discoloured areas round the needle pricks, later longitudinal cracks were formed. The following season these stem and petiole infections were responsible for a number of spots in the leaves in their immediate vicinity. The controls remained healthy.

Cultures of the organism were obtained from the discoloured areas, whose tissues were crammed with bacteria.

2. Portions of diseased tissue were crushed, and the bacteria allowed to diffuse in sterile distilled water. Part of the liquid containing the bacteria was then allowed to stream over both surfaces of

three leaves of a young mango tree. Each leaf was covered for 24 hours with a large glass tube plugged with cotton wool. Characteristic dark angular spots appeared on the inoculated leaves at the end of four weeks.

There were no spots on the controls.

3. Leaves on a third tree were infected successfully in a similar way to that described in experiment No. 2, with the exception that the bacteria were allowed to diffuse in sterile beef bouillon instead of in distilled water.

The controls remained clean.

Inoculation with pure cultures.

4. A young agar streak culture was suspended in sterile distilled water, and the water was allowed to stream over both sides of two leaves of a young mango tree which were covered over for 24 hours as described in experiment 2. A period of cloudy and rainy weather succeeded the date of this inoculation and of those previously described.

Infections were visible on the 21st day, in the form of small, angular, water-soaked looking areas. After a few days these areas began to change colour and rapidly became black. The discoloured tissues were crowded with bacteria, and a pure culture of the specific organism was at once obtained.

There was no sign of infection on the controls.

5. A second experiment was conducted similar to the above, but small punctures were made in the leaf surface with a fine needle. In this case a large proportion of the infections occurred in the neighbourhood of the needle pricks.

Controls pricked with a sterile needle showed no sign of infection.

6. A set of detached mango fruits was washed in 1 : 1000 mercuric chloride, and then in distilled water. They were sprayed with a suspension of a pure culture of the organism and then covered over with a bell jar. After about three weeks there was a small discoloured area round some of the stomata; the blackened areas containing numbers of bacteria. The observation could not be carried further as the fruit was destroyed by *Gloeosporium* sp. No blackening was seen in the controls. Other attempts to infect detached leaves and fruit all resulted in failure, as, in spite of all precautions, they were always attacked by *Gloeosporium* sp. before any infections could show themselves.

7. During the season 1912—13 no inoculations were attempted, but the work was resumed in the spring of 1913. The weather was again

very hot and dry. A young culture was used, a number of young leaves being inoculated on the 13th October by means of needle pricks, and the tree covered over with a wet tent for 24 hours after inoculation. This resulted in a number of small infections chiefly in the region of the needle pricks.

8. A second young tree was inoculated on the 3rd of November. There were a number of very young leaves on the tree, which had not yet lost their red colour; in some of these minute punctures were made with a fine needle and the whole was sprayed with the suspension of a young culture in distilled water. The tree was covered with wet sacks for 48 hours after inoculation. After 27—28 days, there were a few infections on all the younger leaves of the tree, especially in the neighbourhood of the needle pricks and a pure culture of the specific organism was readily obtained from the infected areas.

In both the above experiments the controls were entirely free from disease.

9. Attempts were made in the season 1911—12 to infect some fruits of the "Maroola tree" (*Sclerocarya caffra*) but without success.

Natural methods of infection.

It has been noticed in every instance up to the present that the spread of infection is in the direction of the prevailing winds. It seems probable, therefore, that the wind is the principal agent in spreading the disease and that the organism is carried to a large extent with the dust from beneath the trees. It has been noticed that where the ground is kept free of decaying vegetation and soaked with some germicide, that the spread of infection is to some extent arrested. It has also been observed that when the trees are growing among long grass the infection does not spread nearly so rapidly as when the ground under the trees is bare. This supports the supposition that the infective bacteria are largely carried by the wind.

The younger leaves and the fruit are undoubtedly infected by the rain dripping from diseased leaves. An infected spot is almost invariably found where two fruits are in contact and where a drop of water can easily lodge.

I have noticed very few insects on the trees; they do not appear to feed to any great extent on the mango foliage, and one rarely sees any leaves disfigured by insects. The common stink-bug (*Anoplocnemis curvipes*) is sometimes found on the young foliage, but is probably not responsible to any great extent for spreading the disease.

Morbid anatomy.

Some difficulty was experienced in obtaining good sections, as the tissues, especially of the leaves, are very tough and are apt to tear out of the paraffin. The best results were obtained after fixing in acetic alcohol, which was used hot; and embedding in paraffin in M.P. 60°C. The stain which gave the best result was a combination of Ziehl's carbol fuchsin with light green.

The organism causes no hyperplasias and appears to be entirely confined to the parenchyma. It makes its way into the tissues through a slight abrasion of the surface; it seems very probable that it can also gain an entrance through the stomata, but I have not yet been able to obtain sections which would establish the fact. When once it has effected an entrance, the organism multiplies rapidly and invades the surrounding tissues (Plate VII). The bacteria are very plentiful in the intercellular spaces, and appear to wedge apart the cells and dissolve the middle lamella (Plate IX); one frequently notices in sections through the fruit, a cell which is apparently still untouched, but is completely isolated and surrounded by masses of bacteria.

In some cases the bacteria appear to be intracellular also, but it is almost impossible to judge whether this is really the case or whether the rods have been dragged over the surface of the cells in sectioning; probably the latter.

A good deal of gummosis takes place during the destruction of these cells, the walls of which become swollen and discoloured. The discolouration does not appear to be due to any staining caused by the bacteria but to the decomposition of the cells which are attacked.

The swelling of the disorganised cells is so marked that even in the leaf the surface of the affected area appears raised to the naked eye. The increased thickness of the diseased region is well shown in the section photographed in Plate X. In the fruit this is even more marked and so also is the exudation of gum from the diseased tissues.

The nuclei of the cells in the affected area are abnormally large, and stain deeply with the fuchsin; they are very conspicuous in sections through young infections on the fruit.

As the tissues become disintegrated and the cells killed, the bacteria disappear from the dead areas and are found in more deep-seated tissues (Plate VIII). They do not appear to be capable of attacking lignified tissues—in a number of sections examined there was no trace of bacteria in the fibro-vascular bundles of fruit or leaf, although the surrounding spaces were crowded with bacteria, numbers of which were lying in close contact with the lignified walls.

In the section represented in Plate VIII a number of yeasts were visible in the broken down cells of the surface tissues, but were not present in very great numbers.

For this reason, the infections in the stem very soon cease to increase in size, and can only take place near the growing tip. The diseased spots in the leaf are bounded by some of the smaller veins.

Morphology of the organism. Dimensions.

The organism is a short rod with rounded ends, varying considerably in size on different media.

In the host plant it measures from ·8μ to 2·6μ, by ·5μ to ·7μ. The majority are 1·5 to 1·8μ by ·6μ.

In a preparation made from a 24 hours old culture on nutrient agar (− 15 Fuller) and stained with aqueous Gentian Violet, the limits of length were ·9μ and 2·7μ; the breadth varied from ·5 to ·7μ. The average was 1·5μ × ·6μ. The following are some actual measurements:

1·5μ × ·6μ
1·6μ × ·6μ
2·24μ × ·6μ*
1·4μ × ·5μ
1·4μ × ·6μ
2·4μ × ·7μ
1·6μ × ·6μ
2·3μ × ·6μ*
2·7μ × ·6μ*
·9μ × ·6μ

The rods marked * were just about to divide, and the last in the list was the result of a recent division.

In beef broth cultures 21—24 hours old, short rather thick forms predominate; the rods measure ·9 to 1·4μ × ·6 to ·8μ. The following are some actual measurements taken from a preparation stained with carbol fuchsin:

2·1μ × ·8μ
·9μ × ·8μ
·9μ × ·7μ
1·4μ × ·8μ
1·7μ × ·6μ

In gelatine cultures the rods are exceptionally small, the majority measuring ·9 to 1μ by ·5 to ·6μ. In slides made from a gelatine plate culture three days' old, the longest observed were only 1·6μ.

In solutions containing large percentages of sodium chloride, e.g., beef broth containing 7 to 8·75 % NaCl the organism grows out into long threads which are very variable in length and thickness (Plate XIII, fig. *a*). They are not septate, and a drop from a beef broth culture containing 7 % NaCl examined with the paraboloid condenser showed several of these long filaments in fairly active motion. Some of the longest ones are swollen irregularly and might perhaps be classed as 'involution forms.' They are again mentioned later under this heading.

The following are some measurements of such filaments from a preparation made from a three days' old nutrient broth culture containing 8·5 % NaCl.

32μ × 9 μ – ·6μ
12·6μ × ·6μ
5·9μ × ·9μ

1·9μ × ·6μ
16μ × ·6μ
50μ × ·9μ

In cultures containing 7 % NaCl, the majority of the rods did not exceed 15μ in length, but in the higher percentages there were large numbers of very long filaments.

Fission.

The method of fission was studied in an agar hanging block culture (1, p. 110). The smear on the block was made from a suspension in water of a four days' old culture in 2 % dextrose broth. During the observations the rods were exposed to a brilliant light from a Nernst microscope lamp. The temperature of the room was 25° C., but in the vicinity of the microscope varied from 27° to 30° C. owing to the heat from the lamp before mentioned. The lenses used were $\frac{1}{12}$ mm. Zeiss immersion and No. 12 compensating ocular; with these the changes in the shape and grouping of the organisms could be quite easily followed. Observations were made every five minutes and drawings with the camera lucida about every fifteen minutes.

At 11.15 a.m. two individuals were singled out for study which were lying close together and were apparently the result of the division of a single rod (Plate XII, fig. *a*).

For the first 1½ hours, there was very little change beyond a slight increase in the length of both rods. At 12.55 there was a slight constriction in the middle of one of them (fig. *b*); a transverse wall was formed and at 1.10 p.m. the division was complete (fig. *c*). The second rod increased to nearly three times its original length and then divided in a similar way (figs. *f*, *g*). After this, all the individuals under observation continued to divide rapidly; about 15 minutes elapsed from the first sign of constriction to the completion of division and in another 20 minutes the two new rods thus formed had attained their maximum size and had begun to divide again. The segments were not always of equal size.

Each rod as soon as it divided, pushed away and became completely separated from its neighbour. By a careful adjustment of the illumination, a delicate capsule could be detected surrounding each and preventing close contact.

The small colony which was being formed from the two rods originally selected was kept under observation until 4.30 p.m., when it consisted of 28 rods. At this time other colonies on the agar hanging block numbered 2—24 individuals. In most cases these were the result of the division of a single rod.

At 9 a.m. on the following morning the colonies were easily visible to the naked eye. Under the low magnification their structure was grumose.

Development in the agar hanging block was comparatively slow, doubtless owing to the fact that growth was anaerobic and that for part of the time took place under very strong illumination. After 24 hours the colonies appeared to the naked eye as small white pin spots, whereas a poured plate kept in the incubator at 30° C. for the same length of time developed surface colonies 5—7 mm. in diameter. The submerged colonies, however, were very little larger than those developed on the hanging block.

Grouping.

In young cultures on agar and gelatine, the rods are usually single, but in liquid cultures they frequently occur in pairs.

In the pellicle on the surface of beef broth cultures, chains are formed; these are composed of elements similar to the single rods and may comprise from 2—40 individuals. The chains are straight or curved (Plate XIV, figs. *a* and *b*) and very easily fall apart. There are no chains in the sediment which forms when the tube is in any way disturbed and the pellicle sinks to the bottom.

The chains are not disposed in any particular way, but the orientation is irregular.

Internal structure.

Young, actively dividing rods stain very evenly, but in the older cultures some changes of structure can be observed.

Rods from a ten days' old culture in 2 % dextrose broth when examined with the paraboloid condenser frequently show one or several highly refractive granules (Plate XIV, fig. *c*). In preparations made from very old agar cultures to test for the presence of spores, some of the rods contained small bodies which stained red by Moller's spore stain.

In the sediment of old broth cultures, the rods stain very unevenly, and are apparently vacuolated. Numbers of them have a large central vacuole with a deeply staining portion at each end. In others, there are several bands of colour across the organism in addition to those at the poles.

Capsules.

It has already been mentioned that a delicate capsule could be detected round rods growing on beef broth agar. This was still more evident with dark ground illumination.

In the ring formed round the tube at the surface of liquid broth (1 × 15 F.) cultures, there are numerous organisms with a very definite capsule (Plate XIV, fig. *d*).

When stained by MacConkey's method, these capsuled bacilli take the stain much more deeply than the others; the capsule is unstained and shows as a colourless ring surrounded by the slimy mass in which it is embedded; the latter takes the stain and appears granular.

Spores.

Cultures of various ages and on numerous media were examined for spores but none were detected.

Motility.

In young cultures on agar and in liquid media, the organism is actively motile. The bacteria move rapidly with a forward screw-like movement which does not continue long in the same direction. The rod after proceeding for some distance turns and darts in a different direction, or its progress is interrupted by tumbling movements or by rotation on its long axis. The motile rods are usually single, but fairly frequently pairs may be seen in motion, or occasionally short chains. These move forward in a sinuous manner.

That flagella are present may be seen by using the paraboloid condenser with a Zeiss apochromatic objective 3 mm. ·95 ap. The number and position of the flagella cannot be ascertained, however, as they are in rapid motion, although it is evident that there are several and that they are peritrichous.

When observed under a cover glass, after a while the majority of the rods make their way to the edge of the preparation or to an air bubble where they remain in feeble motion; the remainder gradually come to rest.

The flagella stain fairly easily by van Ermengen's method. They are three to eight in number, peritrichous, slender and 5—7 times the length of the rod (Plate XIV, fig. *e*). A more satisfactory and simple method of staining was found in one described in detail by Ellis (7). This is a modification of Löffler's flagella stain; it is a very simple method and gives a clear stain on a ground almost free from precipitate. I have failed to stain the flagella by the original method of Löffler, or by Pitfield's method.

Involution forms.

The long threads found in cultures containing large percentages of NaCl may possibly be termed involution forms. Some of these are curved and others are swollen irregularly (Plate XIII, fig. *a*). A drop of ± 15 beef broth was placed on a cover glass, inoculated with rods from a beef-broth culture containing 8·5 % NaCl (Plate XIII) and inverted over a moist chamber. The drops contained a couple of dozen rods of varying length and form; the rod shown to the right of fig. *a* was selected for observation.

In 30 minutes two septae had formed and ten minutes later the rod separated into three distinct lengths (fig. *c*). These two stages were observed with the $\frac{1}{12}$th oil immersion (Zeiss) and No. 12 compensating ocular and were drawn with the camera lucida. At this point, however, the rods moved into a deeper part of the drop and it was not possible to get them into focus with the oil immersion. The observation was therefore continued with the Zeiss objective D and compensation ocular No. 18. The drawings were done freehand as near as possible to the scale of the first two.

The portions of the original filament now divided fairly rapidly into segments of very unequal length, and after three hours they became approximately the normal size of the bacillus (figs. *d*—*g*). At this time, the rods being small and rather numerous, they scattered, and it was impossible to trace them further as they became intermingled with bacilli resulting from the division of other filaments.

Some of the rods divided into much more equal segments than the one selected for observation, and the segments remained in contact longer. Fig. *h* shows an exceptionally long one and two shorter ones which had divided in this way.

The long filaments are not rigid and changes of form are frequently observable through curving in various directions.

The culture was exposed to the light of a Nernst lamp during the period of observation and the temperature of the room was 26—27° C.

The slide was placed in the incubator at 30° C. during the night and was again examined the following morning, 22 hours after the culture had been made. The drop was crowded with rods of normal size; in the centre was a thick clump, cochleate in form, but all the outlying rods were in active motion.

Staining reactions.

The organism stains well with all the ordinary aniline dyes; it stains deeply with dilute aqueous solutions of methylene blue, basic fuchsin, thionine and gentian violet; and still more intensely with carbol fuchsin and carbol gentian violet. The last named seems to be the best stain for general purposes.

The bacillus is not acid fast, i.e. it stains blue by the Ziehl-Neelson method but is Gram-positive, and also stains by Claudius' method which has recently been recommended for use in place of Gram's method (2).

When stained by Neisser's method, the bacilli are light brown, and many of them show a small black granule at each pole which is stained with the acid methylene blue. The rods which showed this reaction had been grown on Löffler's blood serum and incubated at 37°C. for 18 hours.

Cultural characters.

In all cultures made for the observation of morphological and cultural characters, preliminary cultivation was practised as prescribed in the chart issued by the Society of American Bacteriologists (1), except that as growth was very slow at 20°C. the cultures were incubated at 25°C. In describing the topography of the colonies, terms are used as defined by Chester (4) and the references following names of colours are to the numbers of the plates in which the corresponding colours can be found in Ridgway's *Colour Standards and Nomenclature* (12).

Nutrient Agar Colonies. Nutrient agar (+ 15 Fuller) was found to be a suitable medium for general purposes. The most characteristic colonies are developed at a temperature of 25°C. They are visible after 24 hours, when they are shining white, circular bodies 1 to 1·5 mm. in diameter; even at this early stage the margin of some of them is becoming undulate; they are denser at the centre than towards the circumference and, when examined microscopically, are granular in texture. The submerged colonies are minute, irregular in form and granular (Plate XI, fig. *a*).

After 48 hours the colonies are 1—2 cm. in diameter, rather irregular in form and with a lobate margin. There is a dense spot in the centre surrounded by one or two concentric rings. The colour is white by reflected light, and a coppery tint by transmitted light. The colonies do not increase in size after this, but change somewhat in colour and texture up to the fifth day; at this time the colour is maize yellow or buff yellow (IV, *VO—Y*, *f—d*) and the colony is surrounded by rather a heavy margin (Plate XI, fig. *a*); the surface is smooth and shining, and slightly raised.

In thickly sown plates the colonies are irregularly circular, with a less deeply lobed or almost smooth margin.

At 30° C. the form of the colonies is somewhat different; they attain a diameter of 1—5 mm. in 24 hours. They are opalescent by transmitted light, with some concentric rings, finally becoming yellow as at 25° C. The maximum diameter is 6—10 mm. and the colonies are irregularly circular with a heavy margin.

At 37°C. growth is also rapid, the colonies are more opalescent than at 30°C. and the concentric rings are more conspicuous. When mature, the colonies are circular and the margin smooth; the colour is slightly deeper than at the lower temperature. The centre of the colony is very slightly raised, and in some there is a heavier ring about 1—2 mm. from the edge, with radiations towards the margin.

The growth at 20° C. is comparatively slow. Colonies are only just visible to the naked eye after 24 hours when they consist of a dense white spot surrounded by a translucent margin. At this temperature thin, spreading colonies are finally formed 7—8 mm. in diameter. They are irregular in shape, and no definite yellow colour is developed; the colonies remaining a creamy tint (19 *yo—y*).

In old plate cultures, especially those at the higher temperatures, there are numerous X-shaped crystals formed (Plate XI, fig. *b*). In all young cultures when examined microscopically, a swaying movement is noticeable right through the colony, due to the activity of the rods composing it.

Nutrient agar streak. On nutrient agar with a reaction of + 15 Fuller, there is quite a plentiful growth after 24 hours at 30° C. It takes the form of a glistening homogeneous streak along the needle track which is somewhat opalescent by transmitted light, milky white by reflected light.

After 48 hours streaks average $\frac{1}{2}$ cm. in width, but taper gradually from a breadth of about $\frac{3}{4}$ cm. at the bottom of the tube to a point near the top of the medium. The difference in the luxuriance of the growth at different levels is no doubt due to difference in the amount of moisture in the medium. The edge is smooth and there is a homogeneous translucent margin about 1·5 mm. wide all round the growth, the central part has become more dense and granular. In some of the tubes there are small opaque spots in this central portion or else longitudinal lines of a similar nature.

The streaks do not usually exceed 1 cm. in width when growth ceases, but if the surface of the agar is very moist, sometimes a spreading growth is formed which almost covers the surface of the agar. Occasionally there is a tendency to form discrete colonies.

The yellow colour is visible after four days, being similar to that developed in the plates. The edge of the growth also becomes heavier than the central portion. The surface is always shiny, and when the medium is becoming rather dry it looks almost like varnish. A heavy yellow sediment forms at the bottom of the condensation water, and on the surface of it there is a pellicle which adheres to the glass tube on the side remote from the slant agar.

The colour of streak cultures is often a little deeper than that of colonies, and varies from buff-yellow to apricot yellow (IV, 19 *YO—Y d—b*). In old cultures X and Y-shaped crystals are formed starting from the surface of the medium and pointing down into the agar. The surface of the agar becomes whitish. There is no noticeable odour.

Nutrient agar stab. There is very little growth in the depth of the medium, only a thin white line following the needle track. A fairly large round colony is formed on the surface of the agar.

Glucose formate agar. A number of cultures were made on this medium as controls to anaerobic cultures. The growth is similar to that on nutrient agar, but if anything more luxuriant and slightly deeper in colour. Crystals are very frequently formed in this medium.

Mango agar. The growth on agar made from an extract of mango fruit was somewhat similar to that on beef-broth agar, but the culture is cream coloured and never becomes yellow. It consists of a glistening streak along the needle track, with smooth edges and very much raised surface.

Treacle agar. The organism only made a very feeble growth on this medium.

Sol. N. with agar. On Marshall Ward's Solution N. solidified with agar the organism grew fairly well for the first 24 hours, but did not subsequently extend very much. There was no chromogenesis; the streak did not exceed 4 mm. in breadth, and was granular round the edges.

The bacillus grew out into very long threads on this medium, but the filaments did not show the irregularities characteristic of those grown in NaCl solutions.

Nutrient gelatine (+ 15 *Fuller*) *colonies.* When incubated at 20° C., colonies on nutrient gelatine are just visible to the naked eye after 24 hours. After 48 hours the superficial circulars are ½ mm. to ¾ mm. in diameter. They are circular, white and glistening, and the surface being very much raised they have the appearance of small drops of milky water. The submerged colonies are smaller and irregular in outline. All are granular as seen with the microscope under a low magnification.

In three days the surface colonies have increased still further in size; they are circular to ellipsoid, and measure up to 1 mm. The smaller colonies are capitate, the larger ones raised; the margin is wavy and texture coarsely granular; a yellowish tinge is becoming evident, and there is just an indication of liquefaction round the edge of the colonies. The submerged colonies are punctiform to the naked eye; when examined under magnification the majority are spherical, though a few are irregular in outline; they are granular in texture, dense in the centre and thinning out to a pellucid margin.

The surface colonies can be lifted out entire on the point of a needle. When mounted in water and examined microscopically, the bacteria diffuse very slowly from the compact mass, but those which escape into the water are very active.

After seven days, the surface colonies measure 1—3 mm., and submerged colonies ¼—¾ mm. in diameter. The former are very much raised, somewhat moruloid, yellow and with irregular margin; the shape is also irregular.

After ten days, each of the surface colonies is sunk in a little saucer of liquefaction, and after 17 days the gelatine is completely liquefied. The yellow colonies are still entire and floating in the liquid medium, though larger and looser in texture than formerly. The colour is similar to, but slightly deeper than, that developed in the agar colonies.

At 12—15° C. similar results were obtained, but growth was decidedly slower. Colonies were not visible to the naked eye until after 48 hours;

the first distinct evidence of liquefaction (colonies crateriform), was noticed after 14 days, and liquefaction was complete in about 24 days.

Nutrient gelatine stab. A number of stab cultures, made in + 15 nutrient gelatine in test tubes measuring 7 × 1″ and incubated at 20° C., showed the following characters.

After 48 hours at 20°C., the needle track was just distinguishable; a thin line of white growth extending to the bottom of the tube. Three days later the growth in the deep parts of the gelatine was seen to consist of very numerous and minute spherical colonies. On the surface of the gelatine there was a colony about 4 mm. in diameter and rather deep coloured.

In nine days the surface growth had sunk in a little saucer of liquefaction, and under this there was a hemisphere of liquefied, clouded gelatine. Five days later the top of the liquefied portion was 1 cm. in diameter; the surface colony remained entire, floating on the liquefied gelatine. Its centre was deep coloured, but it was lighter towards the margin. The shape of the whole growth was napiform, 1·5 cm. wide at the broadest point. There was a sediment at the bottom of the liquefied portion.

In twenty days from the time of inoculation the growth had extended to the width of the tubes and the liquefaction to a depth of 2 cm. (Plate XI, fig. *c*). The cloudy, liquefied portion continued to increase in size, became infundibuliform in shape (Plate XI, fig. *d*), then extended downwards and finally completely liquefied the gelatine.

The colour of the surface growth was deep chrome (Plate III, 17 *O—Y*, *b*) and that of the sediment light orange yellow. The culture had no distinctive odour.

In tubes inoculated at 25°C. liquefaction commenced after six days and reached the stage shown in fig. *d* in nine days.

Nutrient gelatine streak. Streak cultures on nutrient gelatine incubated at 20° C. form a line of growth about 5 mm. broad along needle track in three days. It is smooth, shining and yellow, and opalescent at the edges. In a short time liquefaction commences at the bottom of the streak, making a groove in the gelatine, and as it continues the melted gelatine runs to the bottom of the tube.

Nutrient gelatine shake. Shake cultures in nutrient gelatine developed very numerous, minute colonies near the surface of the gelatine becoming less numerous towards the bottom of the tube; they are evident after three days. Liquefaction sets in on the sixth day, beginning at the surface of the medium and working downwards until the whole is liquefied.

Löffler's blood serum. Streak cultures on Löffler's blood serum at 37°C. at the end of 18 hours show a shining, yellowish growth along the needle track; after 48 hours this becomes deep chrome yellow, forming a streak about 2 mm. broad with smooth edges; it is shining and not raised above the surface of the medium. No liquefaction is observed in tubes kept under observation for several weeks.

Starch Jelly. There was no growth in starch jelly made with Uschinsky's solution even in tubes kept under observation for several weeks.

Potato. The first evidence of growth on potato cylinders is a slight, shining yellowish growth along the needle track; it then spreads over the lower, moist portion of the cylinder, but on the upper half there is no further growth. The colour is buff yellow (IV). There is no greying or other discolouration of the medium.

Mango. On pieces of mango sterilised by steaming in Roux tubes the organism forms a cream-coloured, spreading growth covering the surface of the medium. The mango was not discoloured.

Cocoanut. This is a good medium for chromogenesis, but a plentiful growth is not obtained unless the medium is moist. At 25°C. and 30°C. growth was just visible in 24 hours, first appearing as a shining streak along the needle track; in 48 hours a glistening, cream-coloured growth covered all the lower part of the cylinder. This was the wettest part of the medium, as the pieces of cocoanut were steamed in Roux tubes whose bulb was filled with water, or in ordinary test tubes resting on a wad of wet cotton wool. In three days the colour was slightly deepened, the water in the bulb of the Roux tubes clouded, and in tubes where the cocoanut was resting on cotton wool, the upper layers of this substratum were slightly yellow. The colour deepened and became buff yellow (IV. 19 *YOI—Y, d*). In the Roux tubes, a yellow sediment formed at the bottom of the bulb, and a ring on the glass just above the liquid. As soon as the medium began to dry up growth ceased, and no further changes took place in the appearance of the culture.

Beet. Tubes containing cylinders of beetroot were prepared in a similar way to those containing cocoanut. The organism grows very vigorously on beet; the whole of the cylinder became covered with a shining, wet-looking growth, the upper part having a granular appearance, and the lower portion being wrinkled. In the Roux tubes a heavy pellicle was formed on the surface of the water in the bulb, and in the straight tubes where the cylinder was resting on wet cotton wool the surface of the latter was covered with a yellow wrinkled growth. The colour was deeper than on most media, varying from light orange yellow to deep chrome (III 17, *O—Y*, *d—b*).

Carrot. This was not a favourable medium, a thin spreading growth covered the moist parts of the cylinder.

Nutrient broth (+ 15 Fuller). The bacillus grows very vigorously in nutrient broth; at 30° C. a slight clouding is visible in 6—8 hours; after 24 hours a ring begins to form above the surface of the liquid, and in 48 hours it is well formed and yellowish in colour. A thin pellicle forms on the surface of the broth, and this sinks to the bottom if the tube is slightly shaken and forms a sediment. After three months the broth becomes almost clear, the ring has dried on the sides of the tube, and all of the pellicle fallen to the bottom.

The ring formed above the surface of the broth is very tough and slimy; it is almost impossible to break it with a platinum needle. Cultures in nutrient broth had no noticeable odour.

In broth containing 2 % dextrose, similar results are obtained, but the clouding is heavier; in a medium containing 6 % glycerine, on the other hand, there is a much less copious growth.

Dunham's solution. The bacillus does not grow well in peptone water, but this medium becomes slightly clouded in 48 hours. There is no pellicle and no sediment.

Litmus milk. The tubes when inoculated were deep lavender in colour (XXXVI) and the controls did not change during the time that they were kept under observation.

The organism grows very slowly in milk tubes, and it was not until the tenth day that there was any decided change. After 12 days at 25° C. the colour was pale lilac (XXXVII); on the 16th day it was flesh pink (XIII), and this change of colour was accompanied by a curd-like coagulation of the casein with a separation of whey.

At 30° C. a similar coagulation took place after the same lapse of time, but the reaction was not so acid, the colour being pale cinnamon pink (XXIX). At these two temperatures no further change of colour took place, nor was there any solution of the curd; but after 25 days the colour was completely reduced in all the tubes.

At 37° C. the medium did not turn pink, but after 12 days the colour was completely reduced. The casein coagulated but there was no extrusion of whey. The curd was then gradually dissolved, and after 27 days all that remained was a clear yellow fluid with a slight bacterial sediment at the bottom of the tube.

Uschinsky's solution. The bacillus would not grow in this solution.

Cohn's solution. A slight clouding took place after three days. No ring or pellicle formed, and there was no sediment.

Beerwort. The tubes were kept under observation for a considerable length of time, but they remained clear.

Potato broth. Abundant growth took place in Appel's potato broth. A ring and pellicle were formed.

Cabbage broth. In cabbage broth the organism caused very heavy clouding; there was not much pellicle, but a fairly wide ring formed, and there was about ½ cm. of sediment at the bottom of the tubes. The upper part of the ring was yellow, but the lower part, which was in contact with the broth, was light salmon orange (11). The sediment was the same peculiar colour.

Beet juice. Very heavy clouding took place in this medium, the ring and pellicle were very well developed in colour, the growth was similar to that formed on solid beet.

Solution N. A fair amount of growth was observed in Marshall Ward's Solution N, the medium clouded, but there was no formation of ring or pellicle.

Physical and Biochemical Features.

In this section of the work, unless otherwise stated, the methods used are those outlined by Eyre (8). Each of the tests was repeated several times, and in no case were the results contradictory, although they varied slightly with the vigour of the culture used. Even where not specially mentioned, each experiment was checked by the use of controls.

Enzyme production. The organism is capable of dissolving the middle lamella of cells in the tissues of the host plant, but it has apparently no action on cellulose.

A series of experiments conducted to test for the presence of various enzymes in cultures of the bacillus, for the most part led to negative results. A brief outline of these experiments follows.

Diastatic enzymes. Four tubes of nutrient broth of optimum reaction were inoculated and incubated for five days at 30° C. The cultures were removed from the incubator on the fifth day, and mixed with equal quantities of starch paste containing 2 % of thymol; they were then placed in the incubator at 37° C. for six hours, and at the end of that time were tested for sugar with Fehling's solution. There was no reaction for sugar.

Cylinders of potato on which the organism had been growing for ten days or longer, when tested with iodine gave the purple red reaction characteristic of amylodextrin, but did not reduce Fehling's solution.

Invertin enzymes. Tube cultures in nutrient broth were prepared and incubated as in the previous experiment. At the end of the incubation period the cultures were mixed with an equal quantity of a 2 % solution of saccharose containing 2 % phenol and allowed to stand for six hours. When tested at the end of this time, it was found that the mixture did not reduce Fehling's solution, therefore there were no invertin enzymes present.

Rennet and lab enzymes. Tubes prepared and incubated as before were heated at 55° C. for 30 minutes, in order to sterilise them without destroying any enzymes which might be present. After this 5 cc. of the culture was run into each of three tubes of sterile litmus milk. No coagulation or change of any kind was observed in these during ten days at 20° C.

Acid production. There is no very marked change in the reaction of any liquid medium in which this bacillus is grown. A series of flasks containing nutrient broth with 2 % lactose, saccharose, glycerine, laevulose and dextrose were inoculated and kept at a temperature of 30° C. for ten days; at the end of that time the cultures were slightly more acid than the controls, but only by 2—5 degrees of Fuller's scale.

A test for organic acids was also made, using 500 cc. of 2 % dextrose broth in which the organism had been growing for ten days. The only reaction obtained was a rather doubtful one for succinic acid.

Alcohol production. The first distillate of a ten days old culture in 2 % dextrose broth was divided into three portions.

To the first was added Lugol's iodine solution, then a little NaOH solution. There resulted a smell of iodoform indicating the presence of alcohol, acetone or an aldehyde. A small quantity of Schiff's reagent was pipetted into the second portion; in the greater number of cultures tested there was no reaction, but in one case a faint pink colour appeared, and there was probably a trace of aldehyde in the culture.

To 10 cc. of the third portion was added 10 cc. of H_2SO_4 and 1 cc. of a 4 % solution of potassium permanganate. On adding Schiff's reagent, after five minutes a decided red colour was developed, indicating the presence of alcohol. A decided reaction for alcohol was obtained repeatedly in the distillate from cultures in 2 % dextrose broth.

Ammonia. Two flasks of nutrient broth were prepared, each containing 100 cc. One of these was inoculated, and both were put in the incubator at 30° C. After ten days, 2 grams of calcined magnesia were added to each flask, and 50 cc. distilled from each. Neither of the distillates gave any reaction for ammonia with Nessler's solution. Ammonia is sometimes produced in media containing a nitrate, but this point will be discussed under the heading "nitrate reduction."

Indol and phenol. Tubes of peptone water in which the organism had been growing for ten days at 30° C. always gave a distinct reaction for indol with sulphuric acid and potassium nitrate, there was no such reaction in the controls.

A culture in a flask containing 300 cc. of nutrient broth was used to determine the presence or absence of phenol. After ten days at 30° C. the contents of the flask were tested for indol and phenol as follows. After adding 50 cc. of HCl, the flask was connected with a condenser and 50 cc. distilled over. The distillate was rendered strongly alkaline with KOH and redistilled. The distillate tested with sulphuric acid and potassium nitrite gave a decided reaction for indol.

The residue, when cold, was saturated with CO_2 and redistilled. This third distillate gave no reaction for phenol with Millon's reagent or with ferric chloride. A control flask, similarly tested, gave no reaction either for indol or for phenol.

Pigment production. It has been stated in connection with the description of the cultural characters of the organism that it is capable of producing a yellow pigment on a variety of media; and that this pigment develops more rapidly at 30°—37° C. than at lower temperatures. The colouring matter is insoluble in water, hot or cold, in alcohol, ether, chloroform or dilute acids.

Colour reduction. The organism was grown in nutrient broth tinted with various coloured substances; a number of trials were made in each, but a typical set of tubes is selected in each case for detailed description. In all the experiments the colour of the control tubes remained unchanged.

Litmus. The reduction of litmus in milk cultures has already been described. The colour is also reduced in nutrient broth with or without the addition of dextrose. In a vigorous culture reduction is complete in 48 hours at 30° C., but with a less vigorous strain of the bacillus the process was much slower and took as long as six days.

In one instance the tubes before inoculation were bishop's purple (XXXVII), in 24 hours the reaction of the tubes was more acid, and the colour scarlet red (I). The following day reduction had commenced, working from the bottom of the tube; the lower half of the broth was light salmon orange (II), and the upper half peach red (1). After three days the upper third of the tube only retained any colour and in five days it was colourless. The only considerable variation in this process was in the length of time which it occupied; the medium in every case became slightly acid and then bleached out from the bottom upwards.

Neutral red. Tubes of nutrient broth tinted carmine (I) with this stain were inoculated and incubated at 30° C. The organism grew well in this medium, and a fairly heavy ring formed round the tube at the surface of the liquid. The cultures were kept under observation for ten days, but no reduction took place. The bacterial ring absorbed the colouring matter and became carmine. Rods from this ring when mounted in water and examined microscopically were faintly stained; there were some short capsuled rods, and some long filaments of rather uneven breadth similar to those found in media containing NaCl.

Rosolic acid. The colour of the medium at the beginning of the experiment was peach red (I). After 24 hours at 30° C. the colour was bittersweet orange (II), and it subsequently passed through buff (III) to light orange yellow (II). In six days the culture was entirely colourless except for the bacterial ring at the surface of the liquid which had absorbed the colouring matter and become orange red. The stain was not in this case sufficiently intense to be evident in the bacilli when they were examined microscopically.

Methylene blue. A number of tubes and fermentation tubes were prepared containing broth tinted with methylene blue to the colour of a strong ammoniacal solution of copper sulphate. The closed arm of the fermentation tube was colourless after sterilisation and remained so throughout the experiment; the description of changes of colour therefore refers to the ordinary test tubes, and to the open arm of the fermentation tubes.

As in the case of tubes coloured with litmus solution reduction commenced at the bottom of the tubes. After the cultures had been kept at 30° C. for 24 hours the lower part of the tube was colourless and the upper part Tyrolite green (VII). On the second day there was only about $\frac{1}{2}$ cm. at the surface of the liquid which retained the green colour. After six days the medium was colourless, but the bacterial ring had partially absorbed the colour and was Venice green (VII).

The colour did not return at all when the tubes were shaken.

Indigo carmine. Indigo carmine was also gradually reduced from the bottom of the tube upwards. In this case the organism did not absorb the colouring matter. Reduction was complete in eight days.

Congo red. Congo red was not reduced, but the dye was absorbed by the superficial bacterial growth as in the case of neutral red.

Cultures were made in nutrient broth tinted with several other dyes, chiefly in order to determine whether any of these would inhibit the growth of the organism. None of them actually inhibited the growth in very dilute solutions, but growth was feeble in broth containing malachite green or cyanin.

The bacillus grew well in Trypan blue, and completely reduced the colouring matter; Trypan red was not reduced, but the surface growth absorbed the stain.

Reduction of nitrates. Nitrates were more completely reduced when the organism was grown in nitrate water than when it was grown in nitrate broth.

A flask of nitrate water (8, p. 169) was inoculated from a young culture of the organism in nutrient broth and incubated with a control flask at 30° C. for five days. The culture was then divided into four portions, the first two being used to test for the presence of nitrites. There was no reaction for nitrites when tested by the iodine-starch method or with α-naphthylamine and sulphanilic acid.

A third portion tested with Nessler's solution developed a distinct yellow colour indicating the presence of ammonia. The remainder of the culture was evaporated to dryness and tested for the presence of nitrates with phenolsulphonic acid. There was no reaction.

The nitrate water in the control flask tested in a similar way gave a distinct reaction for nitrates but none for nitrites or ammonia.

The test for nitrate reduction was repeated using nitrate broth (8, p. 143) in place of nitrate water. The culture and control were tested on the fifth day in the manner described above and as before the control was found to contain nitrates but no nitrites or ammonia. The culture reacted strongly both with iodine and starch paste, and with α-naphthylamine and sulphanilic acid showing the presence of nitrites; there was no reaction, however, for nitrates or ammonia.

Fermentation tubes. The organism was grown in fermentation tubes containing 2 % sugar broth, but there was no gas production with any of the carbohydrates used. In one solitary case gas was formed from dextrose and saccharose, but this proved to be due to the presence of an interloper. The vigour of growth in the open and the closed end varied greatly with the composition of the medium. The intensity of clouding in media containing various carbohydrates is shown in the following table:

Carbohydrate used	Clouding in closed arm	Clouding in open arm
Dextrose	heavy	very heavy
Saccharose	heavy	very heavy
Laevulose	moderate	heavy
Maltose	moderate	heavy
Raffinose	slight	heavy
Galactose	very slight	moderate
Mannite	slight	heavy
Lactose	none	fairly heavy
Dextrin	very slight	very heavy
Glycerine	none	moderate

It will be noticed from this summary, that the organism does not grow anaerobically in the presence of glycerine and lactose, and only feebly in the presence of galactose and dextrin.

Hydrogen sulphide. Tubes of peptone lead solution (8, p. 186) were prepared in order to test for the presence of H_2S. Cultures in this medium were kept under observation for sixteen days, but there was no formation of hydrogen sulphide; the precipitate was not blackened. At the end of sixteen days the cultures were discarded.

Toleration of NaCl. The bacillus can grow in media containing comparatively large percentages of sodium chloride. A number of tubes containing various percentages of NaCl (from ·5 % to 10 %) in + 15 nutrient broth were inoculated from a young culture and incubated at 30° C. Tubes containing less than 4 % NaCl were clouded in 18 hours, while those containing from 4 % to 8·25 % were clouded in 48 hours. In tubes containing 9 % and over there was no growth; in the 8·5 % and the 8·75 % solution the clouding was so slight as to be barely noticeable, but a microscopic examination showed that the organism had multiplied considerably. The abnormal forms found in this medium have been described elsewhere.

Reaction of medium. The organism is not particularly sensitive to the reaction of the medium in which it is grown. The optimum is about + 17 of Fuller's scale, but there is very little variation in the rapidity of growth in media of reactions varying from +14 to + 23 Fuller. During an experiment undertaken to find the optimum reaction, broth tubes having these reactions clouded in six hours at 30° C.; those with a reaction of + 2 to + 14 and from + 24 to + 27 Fuller clouded in seven hours; those with reactions from + 28 to + 30 and from + 10 to + 1 in eight hours. In tubes containing more alkaline broth growth was considerably slower, the organism preferring a medium which is slightly acid to phenol-phthalein.

A series of cultures was made to test the ability of the bacillus to grow in media containing varying quantities of certain acids and alkalis.

To flasks containing 50 cc. of neutral broth were added different percentages of acetic, oxalic, tartaric, and malic acids, and of sodium hydrate and sodium carbonate. With each of these substances a series of flasks was prepared varying in reaction from 0 to ± 50 of Fuller's scale, the intervals between the reactions of any two in a series being five degrees, so that there were ten flasks in each series. The contents of each flask were pipetted into four tubes and these were sterilised. A loopful of a young broth culture was introduced into three tubes of each set and the fourth kept as a control. The results obtained from the observation of these cultures may be tabulated as follows, the controls in every case remained clear.

Substance tested	Amount to retard growth	Amount to inhibit growth
	Reactions in terms of Fuller's scale	
Acetic acid	+ 25	+ 30
Oxalic acid	+ 30	+ 35
Tartaric acid	+ 35	+ 40
Citric acid	+ 30	+ 40
Malic acid	+ 35	+ 35
Sodium hydrate	– 30	– 45
Sodium carbonate	– 30	– 35

Atmosphere. That the organism is a facultative anaerobe was suggested by the fact that it grew in the closed end of fermentation tubes and in the depth of the medium in stab cultures, though in the latter growth is never abundant. A series of cultures was therefore made to test the ability of the bacillus to grow in the absence of oxygen and in the presence of various other gases. Bulloch's apparatus was used in making most of the anaerobic cultures, and the method used in each case was similar to the one now to be described; slight variations in method were of course necessary in order to introduce the various gases into the apparatus, but these are mentioned in connection with the individual experiments.

Oxygen and carbon dioxide absorbed. Streaks were made on slant nutrient agar and glucose formate agar, and a loopful of a young broth culture introduced into tubes of nutrient broth and glucose formate broth. These were placed in a beaker standing in a glass basin at one side of which was placed a small heap of dry pyrogallic acid. The whole was next put inside the Bulloch's apparatus, the bell jar carefully sealed on to the ground glass plate and the air partially exhausted from the apparatus by attaching the exit tube to a suction pump. A strong solution of KOH was then siphoned into the glass basin without admitting air, dissolving the pyrogallic acid. A strong alkaline solution of pyrogallic acid was thus formed which absorbed the oxygen and carbon dioxide within the bell jar.

The apparatus was kept at a temperature of 30° C. with a number of control tubes. In 48 hours there was a very vigorous growth in all the control tubes; the growth in the tubes deprived of oxygen was exceedingly slow as compared with that in the controls, and was not at all abundant.

This experiment was repeated a number of times with similar results.

Hydrogen. A set of cultures was prepared and placed in the Bulloch's apparatus as described in the previous experiment. In this case, however, hydrogen was generated, and after being purified by passing through wash bottles containing solutions of silver nitrate, potassium permanganate and potassium hydrate, it was allowed to stream through the apparatus until all the air was expelled. Any oxygen or carbon dioxide remaining in the bell jar was absorbed as before with an alkaline solution of pyrogallic acid.

The growth in the tubes in the atmosphere of hydrogen was slightly better than in those kept in an atmosphere composed chiefly of nitrogen. This was found to be the case in two successive experiments.

A set of tubes inoculated from hydrogen cultures and grown in Buchner's tubes in the absence of oxygen made better growth than the cultures deprived of oxygen which had been made direct from aerobic cultures.

Carbon dioxide. The apparatus was prepared in the same way as before and a current of carbon dioxide passed through it. In this case any remaining oxygen was absorbed by means of a solution of pyrogallic acid in water. There was a slight growth in all the tubes at the end of 48 hours, but not quite so much as in those grown in an atmosphere of hydrogen. Growth was abundant in all the control tubes.

Sulphur dioxide. In this case SO_2 was passed through the apparatus until all the air was displaced before the introduction of the KOH solution. There was no growth in any of the tubes, nor did any growth take place when the tubes were removed from the apparatus and placed in the incubator, the organism having been killed by prolonged exposure to the gas.

The ability of the bacillus to withstand short exposures of SO_2 was also tested. Transfers were made from a young culture to ten tubes of slant agar, a generous quantity of the culture being used. Two were placed in the incubator as controls, and the remainder were exposed to a stream of SO_2 for periods varying from 15 seconds to five minutes. In tubes exposed for 15 or 30 minutes there was a slight growth along the needle track after five days; in those exposed for longer periods the organism was killed. Growth was abundant in the controls.

A drop of a liquid culture was introduced into each of eight tubes of nutrient broth, and a stream of SO_2 passed through the tubes for periods varying from 15 to 60 seconds. There was no clouding in any of these.

Reduced pressure. A set of tube cultures was prepared and sealed in Bulloch's apparatus; then the air was exhausted as completely as possible from the bell jar. In this experiment the oxygen was not absorbed.

At the end of four days there was only a slight growth in the glucose formate agar and in the formate broth tubes, but quite a good growth in the tubes containing ordinary nutrient agar and broth.

Effect of germicides. In connection with the spraying experiments a series of cultures was made to determine the susceptibility of the organism to various germicides.

In testing substances which could be added to nutrient broth without causing precipitates the procedure was as follows: A young broth culture was used, one which had been kept at 30° C. for 24 hours. From this ·1 cc. of the culture was dropped into each of a number of tubes of + 15 broth, and this amount did not cause any clouding of the medium. Into the tubes thus inoculated, each of which contained exactly 10 cc. of broth, varying quantities of a solution of the germicides were pipetted, about five tubes being used for each percentage.

After remaining in the incubator at 30° C. for 30 minutes a series of plates was poured from one tube in each group, the remaining four being returned to the incubator. The plates and tubes were kept under observation for a number of days until it was certain whether growth would take place or not.

Only a few substances could be satisfactorily tested in this way, and the results obtained with these may be tabulated as follows:

Germicide	Amount to retard growth	Amount to inhibit growth	Amount to kill organism in 30 mins.
Formalin	1 : 20,000	1 : 10,000	1 : 1,000
Copper sulphate	1 : 1,500	1 : 1,000	1 : 200
Phenol	1 : 1,500	1 : 1,000	1 : 100
Sodium nitrate	1 : 25	1 : 10	
Kainite	1 : 25	1 : 10	

Lithium sulphate in the proportions of 1 : 25 had no effect in retarding growth. I did not find any involution forms in cultures containing 4 % of this salt.

Substances which caused precipitates in nutrient broth were tested in a slightly different way. Tubes were prepared containing sterile distilled water with various percentages of the germicide. Into these ·1 cc. of a 24-hour old broth culture was pipetted and they were allowed to stand at 30° C. for thirty minutes. Plates were then poured as from the beef broth tubes in the previous experiment and these were kept under observation for several days.

The results obtained were as follows:

Germicide	Amount to kill large percentage of organisms in 30 mins.	Amount to kill all organisms in 30 mins.
Mercuric chloride	1 : 20,000	1 : 10,000
Hycol	1 : 2000	1 : 1000
Formalin	1 : 2000	1 : 1000
Cyllin	1 : 2000	1 : 1000
Lysol		1 : 400
Copper sulphate		1 : 400
Potassium permanganate	1 : 400	1 : 350
Iron sulphate	1 : 100	1 : 25
Iron sulphide	1 : 75	1 : 25

From this table it is evident that the organism is most susceptible to mercuric chloride, but it was not considered advisable to make use of this substance for spraying owing to its highly poisonous nature.

The results of spraying with Hycol have been given in another part of the paper.

Action of sunlight. The organism is not sensitive to exposure to light. It grows well in the diffused light of the laboratory, though not so rapidly as in the dark.

A series of thinly sown plates were exposed to direct sunlight, any undue heating being prevented by placing over each a glass basin containing about 2 cm. of a 2 % solution of potash alum. The number of colonies on plates exposed one to four hours was not appreciably less than the number developed in the control plates; there was a slight diminution in numbers in those exposed for five hours. This experiment was conducted in winter during the month of July.

Four cover glasses on which a film of bacilli had been dried were exposed to bright sunlight for one, two, three and four hours, then dropped into tubes of nutrient broth. Those exposed for one and two hours clouded the broth in 24 hours; those exposed three and four hours in 48 hours.

Thermal Relations.

The apparatus used for the determination of the thermal death point and figured below was adapted by Mr Ensor of the Public Works Department from a Hearson vacuum embedding bath intended for working with gas; it answers the purpose admirably, and regulates within one-tenth of a degree.

I am indebted to Mr Ensor for the following brief description of the apparatus and for the figure explaining the electrical connections:

Constant temperature water bath. The apparatus consists of the water bath of a Hearson vacuum embedding bath (*A*, diagrams 1 and 2). This is electrically heated by means of two eclipse elements (*L*, diagram 3) fixed to the bottom of the bath. The elements are connected in series on 250 volts.

For automatic regulation of temperature a switch (*B*, diagrams 1, 2 and 3) actuated by a capsule (*J*, diagram 3) is provided, this makes or breaks the heating circuit at the required temperature. A lamp (*K*, diagram 3) is joined across the switch contacts to prevent sparking.

There is also a tumbler switch (*C*, diagrams 1, 2 and 3) by means of which the temperature can be controlled by hand if desired.

The water is continually stirred by means of a paddle (*D*, diagrams 1, 2 and 3) driven by a small motor *E* fixed above the bath.

In order to cut down the speed of the motor to about 60 revolutions per minute a lamp (*H*, diagram 3) is connected in series. The speed can be varied by placing lamps of different candle power in the lamp holder.

The motor is started and stopped by the switch *F*.

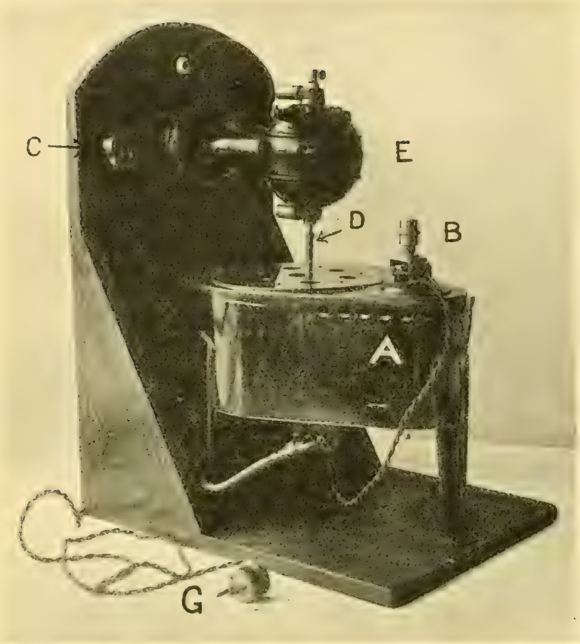

Fig. 1

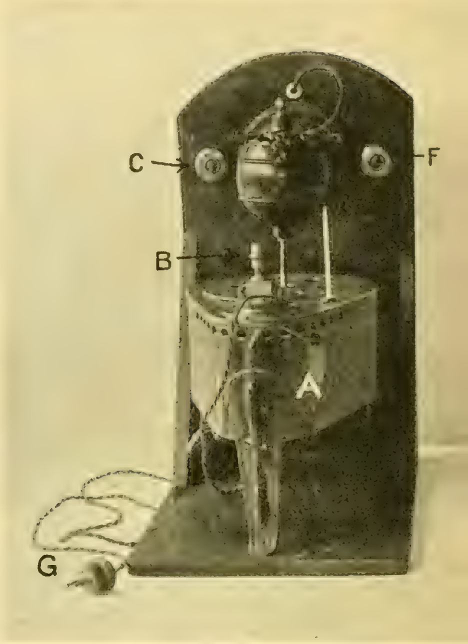

Fig. 2

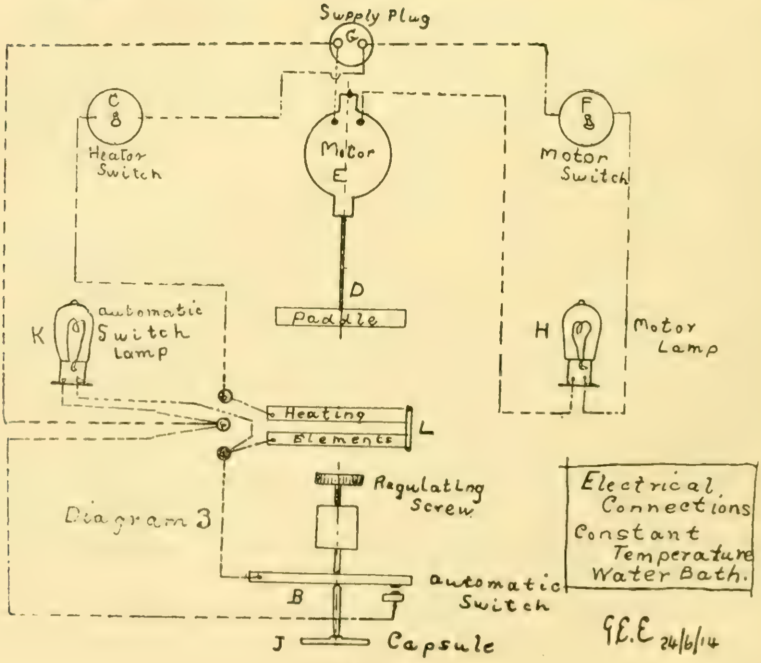
Supply Plug
G
C
Heater Switch
Motor
E
F
Motor Switch
D
Paddle
K
automatic Switch lamp
H
Motor Lamp
Heating
Elements
L
Regulating Screw
Diagram 3
automatic Switch
B
J
Capsule
Electrical Connections
Constant Temperature Water Bath.
G.E.E. 24/6/14

Fig. 3

The apparatus is connected to the nearest supply plug or lamp holder by means of a flexible cord and plug or adapter.

The tests were made with specially uniform tubes of thin glass, about 18 cm. in diameter and containing exactly 10 cc. of nutrient broth. Into each of these was introduced a loopful of a 24-hours old culture in nutrient broth, and they were placed in the water bath for ten minutes; at the end of the ten minutes the tubes were plunged into cold water to reduce the temperature and were finally placed in the incubator at 30° C. with a number of control tubes. The thermal death point was found to be 60° C. when determined by this method.

A second series of experiments was undertaken in order to determine the death point of the organism in a dry condition.

A number of sterile cover slips were smeared with a suspension of an agar streak in a normal saline solution. These were dried in a sterile petri dish and then exposed for ten minutes to the heat of a drying oven. At the end of this time they were taken from the petri dish with a pair of sterile forceps and dropped into tubes of nutrient broth. The broth clouded when inoculated with cover slips exposed to temperatures of 120° C. and under, but remained clear if the cover slips dropped into it had been exposed to a temperature of 125° C. or over. The thermal death point of the organism in a dry condition, therefore, lies between 120° C. and 125° C.

The organism can grow through a wide range of temperature if it is provided with sufficient moisture. It grows very slowly at 5—6° C. and also at 45°. At the latter temperature nutrient broth was feebly clouded at the end of 48 hours. The optimum temperature is about 30° C.

Desiccation.

The organism will not grow without a fair amount of moisture, and its growth is most vigorous on a wet medium and in a saturated atmosphere; on the other hand, it is very resistant to desiccation and remains alive for a long time in a dry condition.

A number of diseased leaves were dried in the air in the laboratory, being merely protected from dust. They were received on the 9th of June, 1913, and from that date cultures were made from them at intervals of a month. On the 9th of June, 1914, after the leaves had been drying for one year a vigorous culture was obtained; it has not yet been ascertained how much longer the organism retains its vitality on dried leaves.

An experiment was also conducted to test the ability of the bacillus to withstand desiccation on glass cover slips.

A 48-hours old culture on nutrient agar was suspended in a normal saline solution and a number of sterile cover slips smeared with the suspension were put in a sterile petri dish to dry.

A Hempel desiccator containing sulphuric acid was prepared, the dry cover slips transferred to a sterile ventilated capsule, and placed in the desiccator. The latter was then sealed up and partially exhausted. At intervals cover slips were removed from this apparatus with sterile forceps and dropped into tubes of nutrient broth. After 40 days drying a culture of the organism could be still obtained from the cover slips; at the end of that time the experiment was discontinued.

RESUMÉ OF SALIENT CHARACTERS.

Bacillus mangiferae n. sp. An organism causing a disease of the leaves and fruit of the mango (*Mangifera indica*); it attacks the parenchyma, causing black angular spots on the leaves; and on the fruit discoloured roughened areas often accompanied by deep longitudinal cracks.

A short motile rod with rounded ends averaging $1{\cdot}5 \times {\cdot}6\,\mu$; usually single, chains formed in pellicle on liquid media; motile, with 2—8 long, peritrichiate flagella; no spores observed; capsules in ring above liquid media; involution forms (?) in broth containing high percentages of NaCl; stains readily with usual stains and by Gram's method.

Forms shining yellowish colonies on nutrient agar, undulate at 25° C.; liquefies gelatine; clouds nutrient broth forming ring pellicle and sediment; grows readily on blood serum, but does not cause liquefaction; grows slowly in milk finally causing coagulation, at 37° C. the casein is slowly dissolved. Grows on potato making a shining yellowish spreading growth over moist part of medium; the latter is not discoloured; heavy growth on beet and in beet juice, also in cabbage broth; slightly clouds Cohn's solution; no growth in Uschinsky's solution or on starch jelly made with Fermi's solution.

No gas formation in carbohydrate media, but medium becomes slightly more acid. No formation of diastatic or invertin enzymes, small percentage of alcohol in dextrose broth; nitrates reduced, tolerates up to 8·75 % NaCl; reduces litmus and several other colouring matters; indol in media containing peptone but no phenol.

Aerobic, facultative anaerobe; very sensitive to action of mercuric chloride, but less so to copper sulphate; not sensitive to sunlight; will not grow without moisture, but is not easily killed by desiccation; will grow in media of widely different reaction, optimum reaction about + 17 Fuller; maximum temperature for growth 45° C., thermal death point 60° C. Group No. 221. 2223532.

SUMMARY.

1. A disease of mangoes, hitherto undescribed, has for the last few years been causing considerable loss to mango growers in the Union.

2. So far as can be ascertained the disease is not known outside South Africa, and it occurs in this country in the neighbourhood of Barberton and Warmbaths, in the coast region of Natal and at Delagoa Bay.

3. Dark angular spots are formed on the leaves which do not noticeably affect the general health of the tree, but serve as a source of infection for the fruit. On the latter the disease causes discoloured roughened areas and deep cracking; infected fruit is detached from the tree by the slightest air movement and falls rotting to the ground.

4. Infection is carried by the wind, and by rain dripping from infected leaves. Very few insects are found on the mango foliage.

5. Spraying experiments have been conducted which show that spraying with Bordeaux mixture, iron sulphide or Hycol is useless in checking the disease.

6. The cause of the trouble is a flagellate bacillus *Bacillus mangiferae* n. sp. It invades the parenchyma, wedging apart and killing the cells and causing gummosis; lignified tissues are not touched.

7. The organism is described in detail and a resumé is given of its salient characters.

ACKNOWLEDGMENT.

I wish to acknowledge my indebtedness to Mr I. B. Pole Evans, Chief Division of Botany, in whose laboratory the work was done, for many helpful suggestions; and to Mr E. E. Ensor of the Public Works Department for adapting the apparatus described in connection with the thermal death point determinations.

The names of the varieties of mangoes grown in this country were furnished by M. D. d'Emmerez de Charmoy, of the Department of Agriculture, Mauritius.

I have also to thank Messrs Winter Brothers, of Barberton, who placed their orchard at our disposal for the spraying experiments, and who have repeatedly supplied us with mango fruit for experimental purposes.

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EXPLANATION OF PLATES.

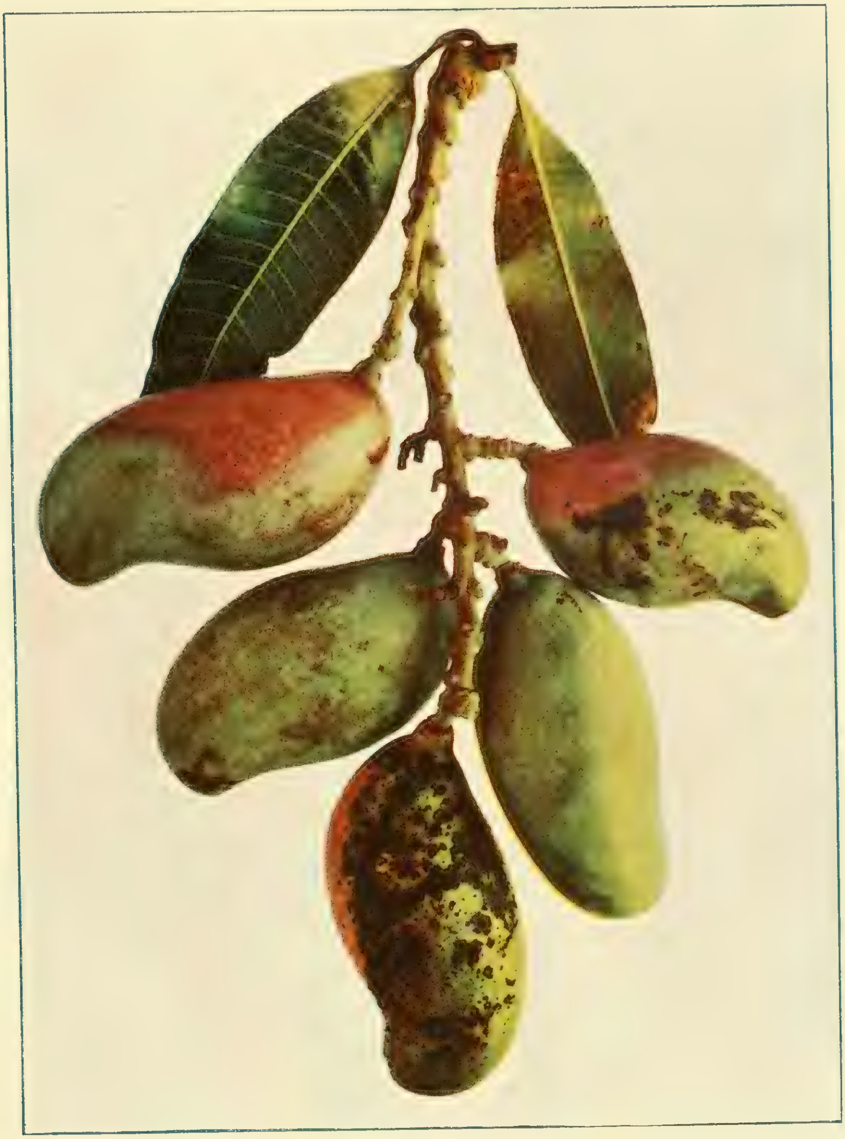
All drawings were made with the aid of the camera lucida, except figures *c—h* of Plate XI, which were drawn freehand as explained in the text.

Plate I. Three colour photograph of small branch bearing diseased fruit. (Reproduced, from nature, by the Government Printer, Pretoria.)

Plate II. Mango leaves showing the dark coloured, angular spots, characteristic of the disease.

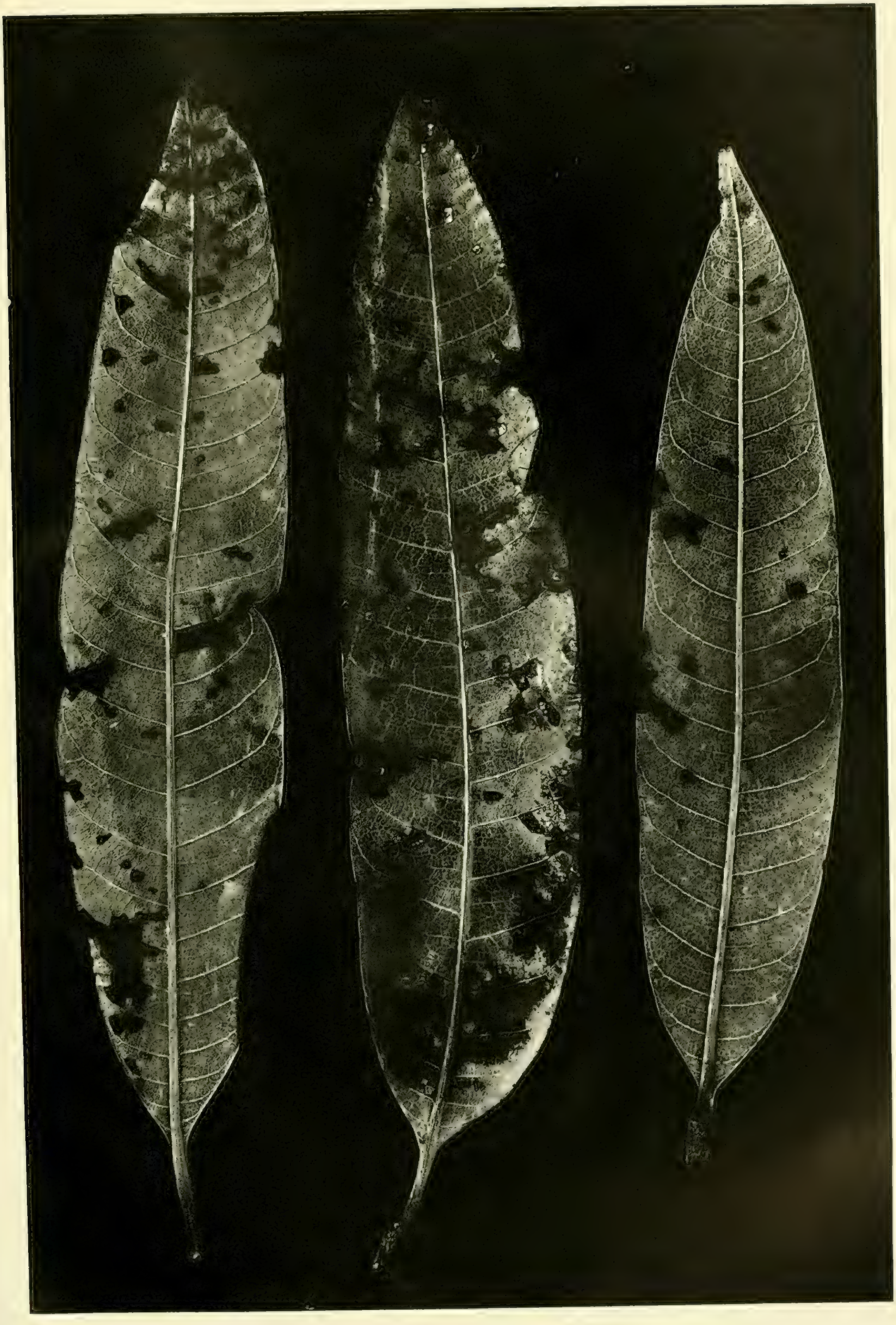
Plate III. Small branches which have been infected through hail injuries. A small portion of bark has been removed from the left-hand branch to show the discolouration of the underlying tissues.

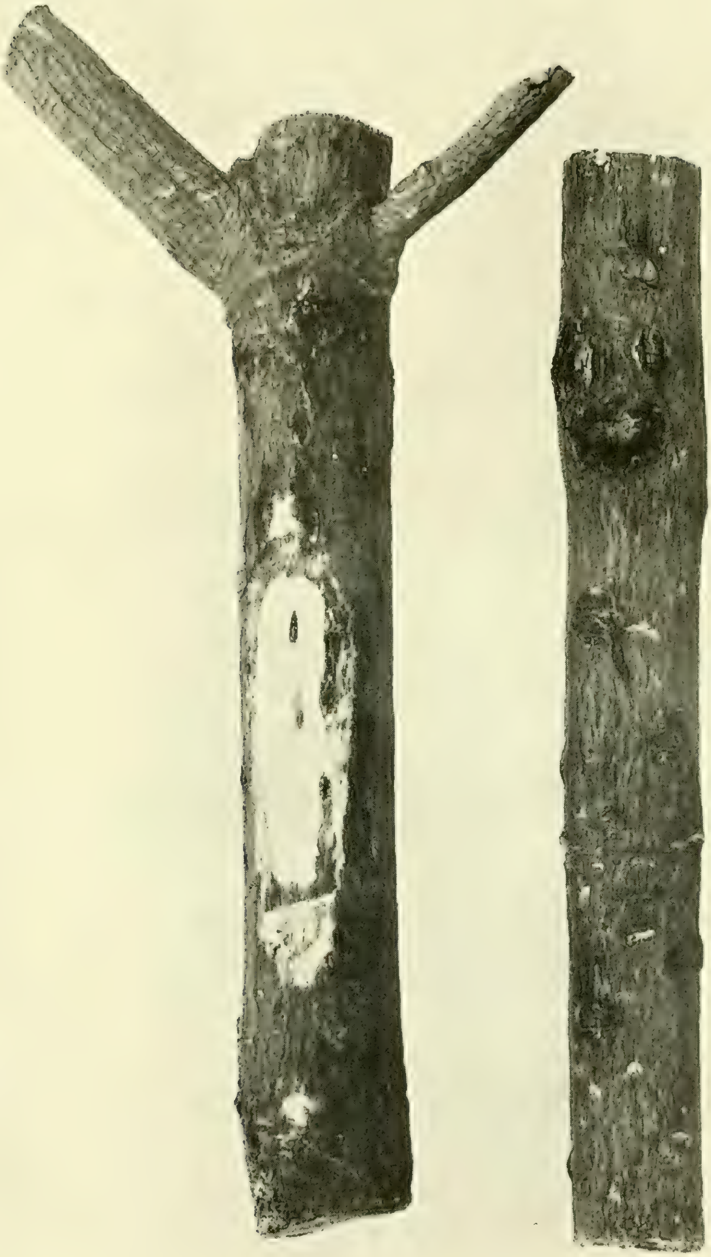
Plate IV. Half-grown fruit showing discoloured roughened areas caused by *Bacillus mangiferae.*

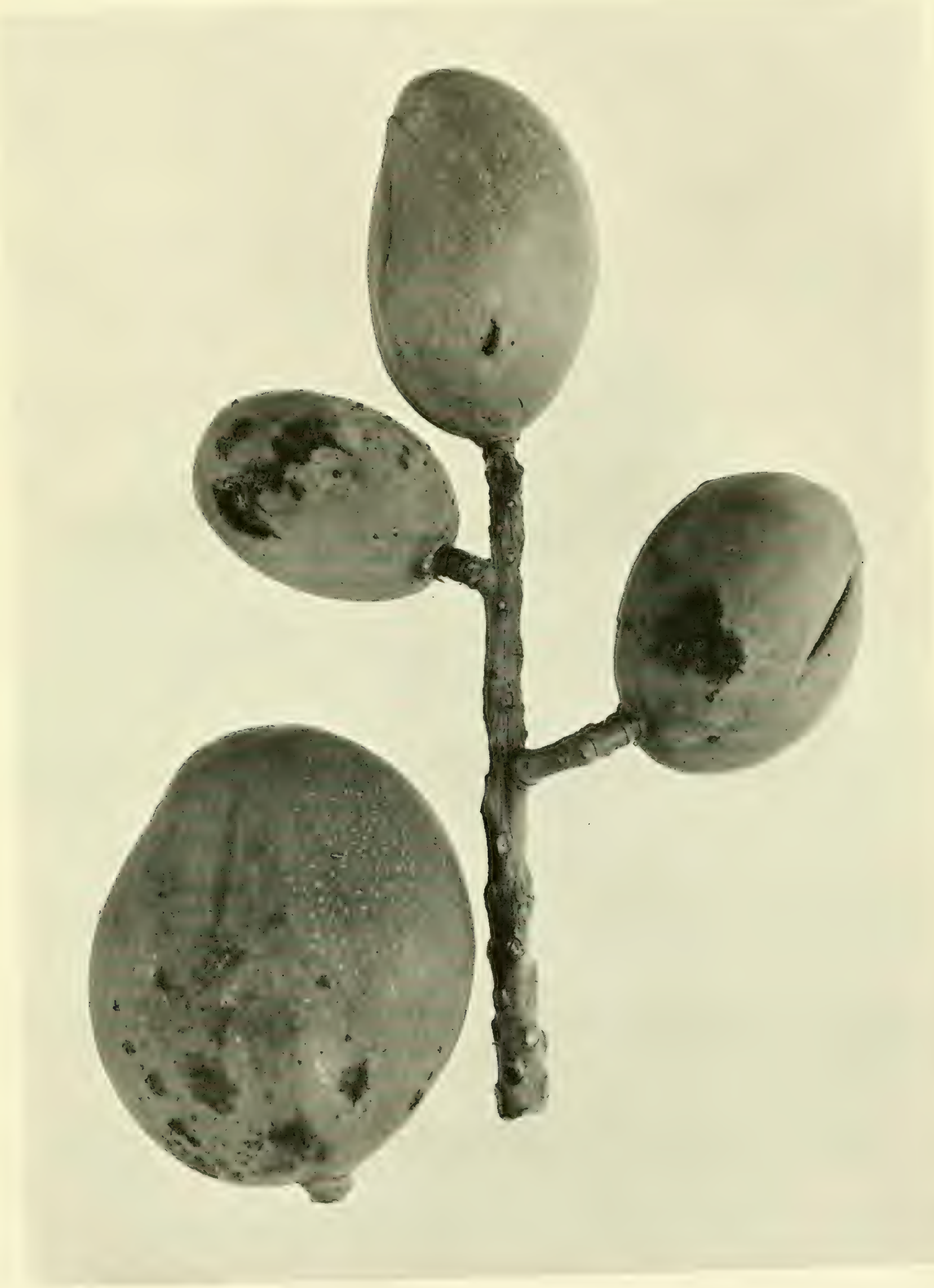


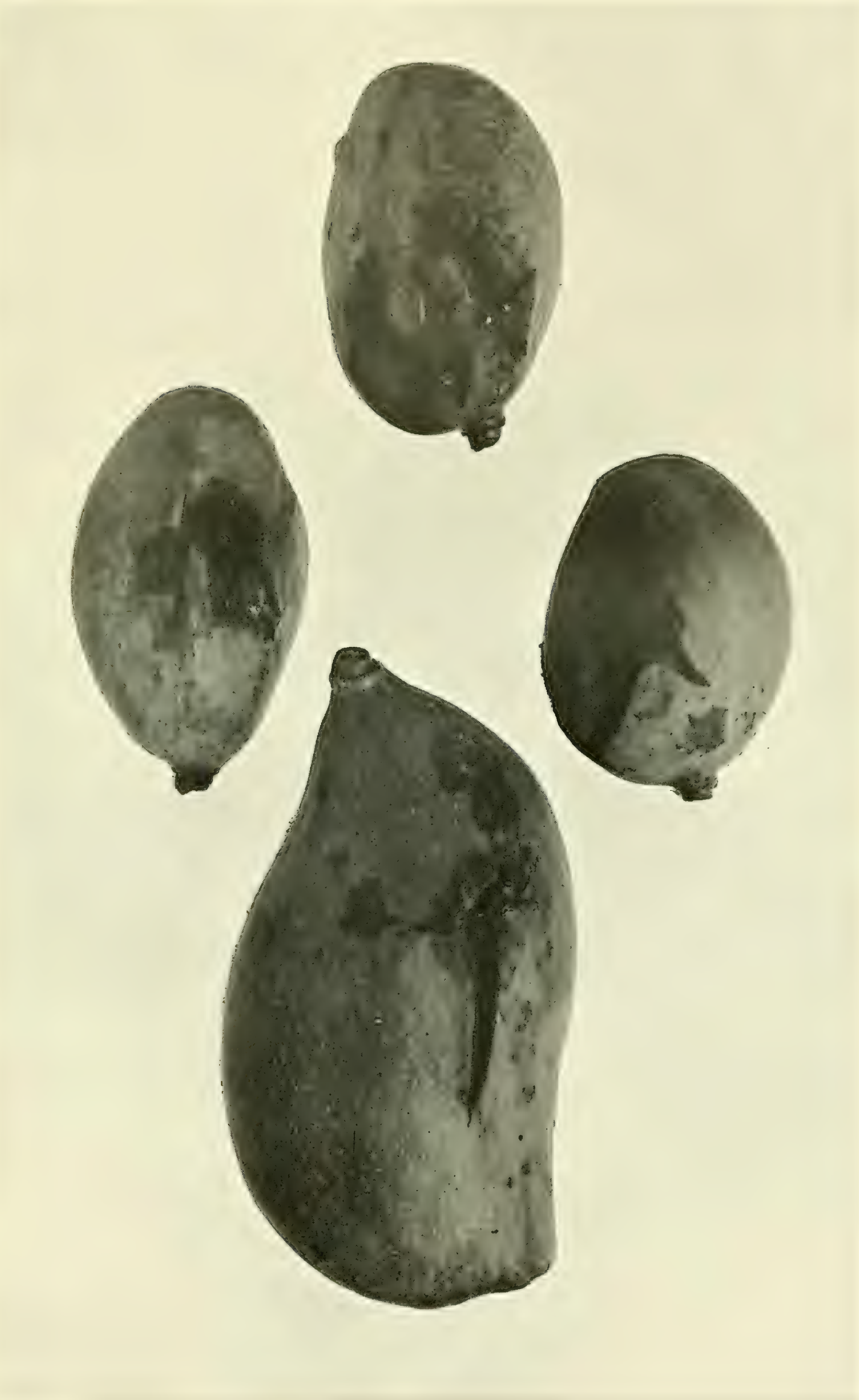
Reproduced, from nature, by the Government Printer, Pretoria

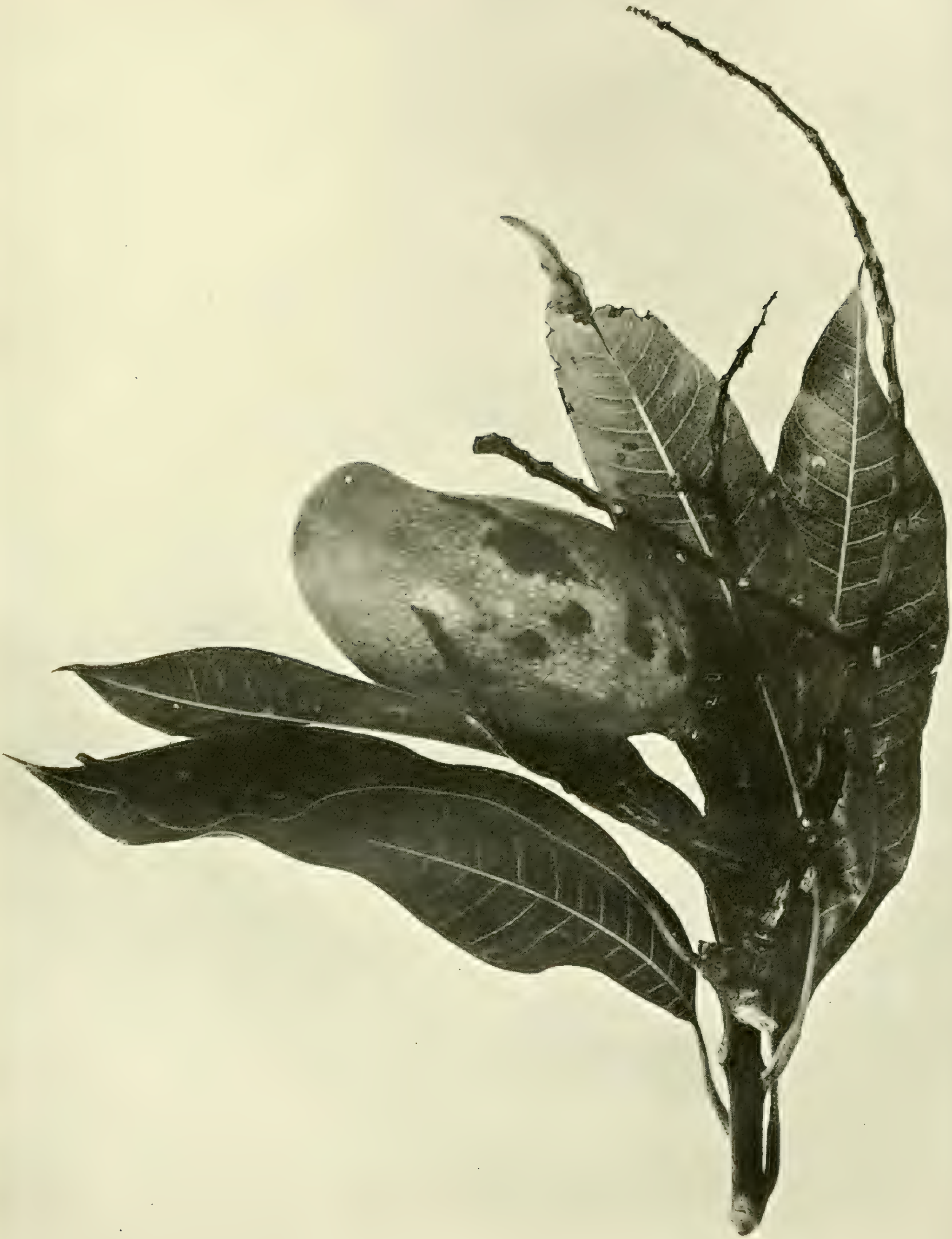
Mango fruits, attacked by Bacillus mangiferae

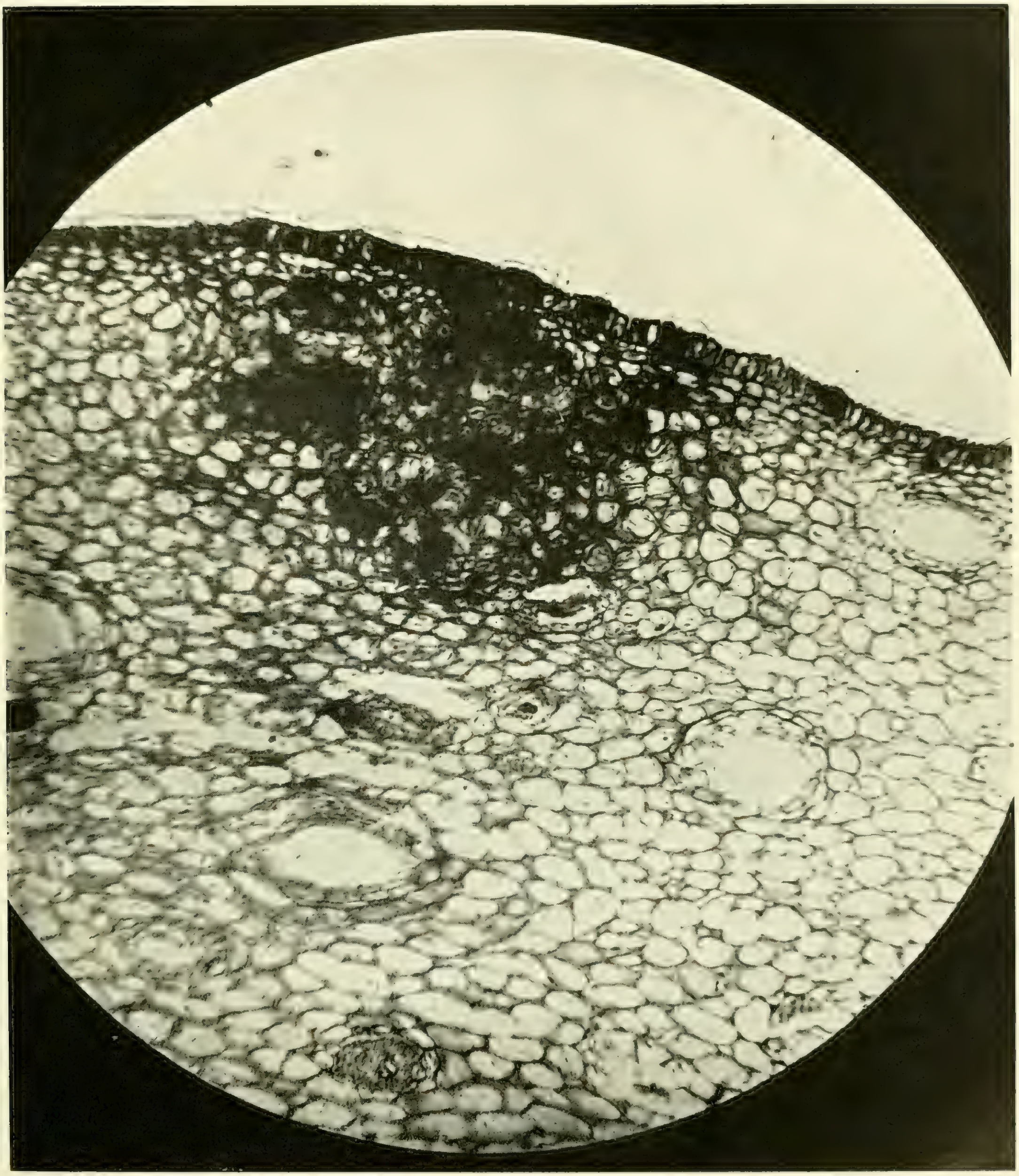


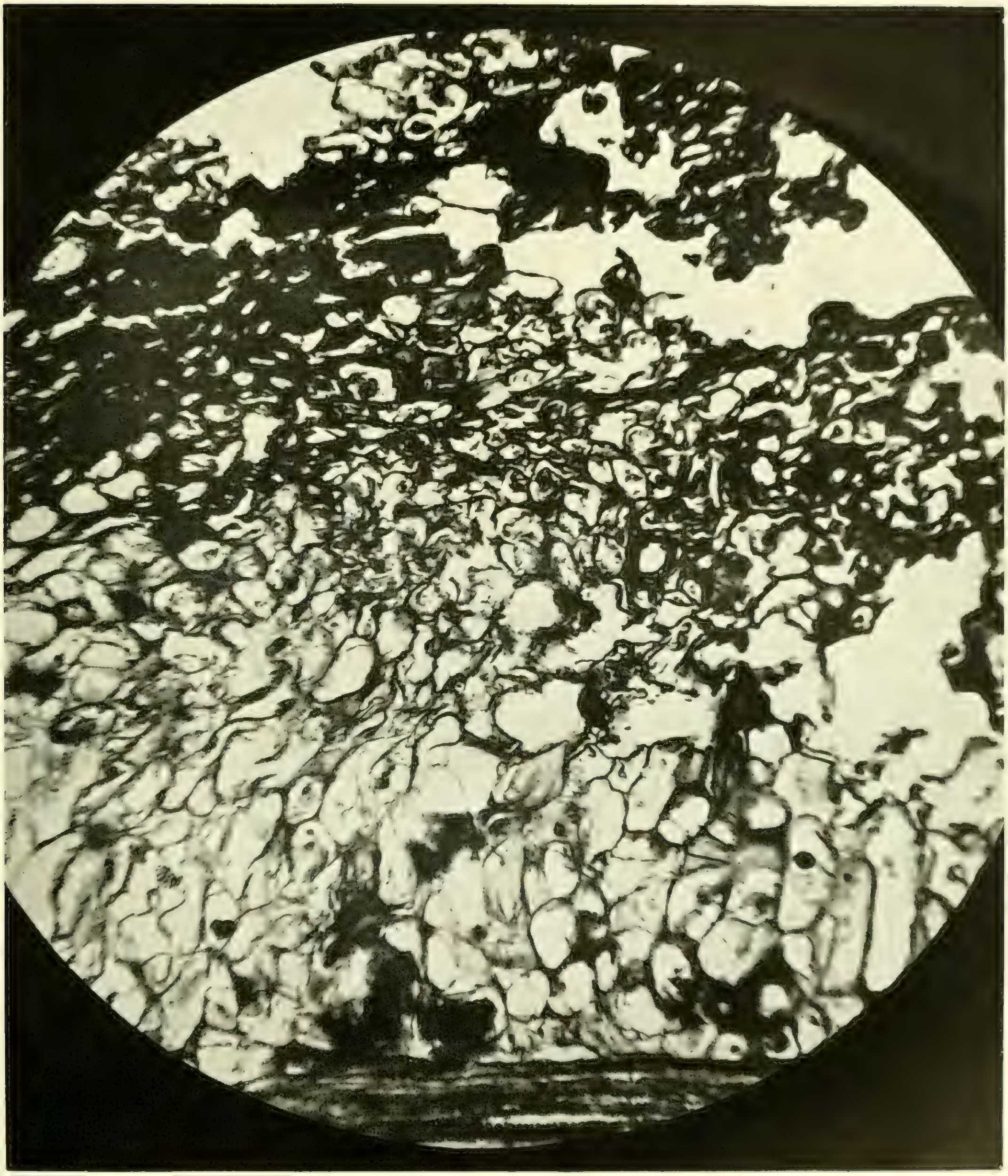


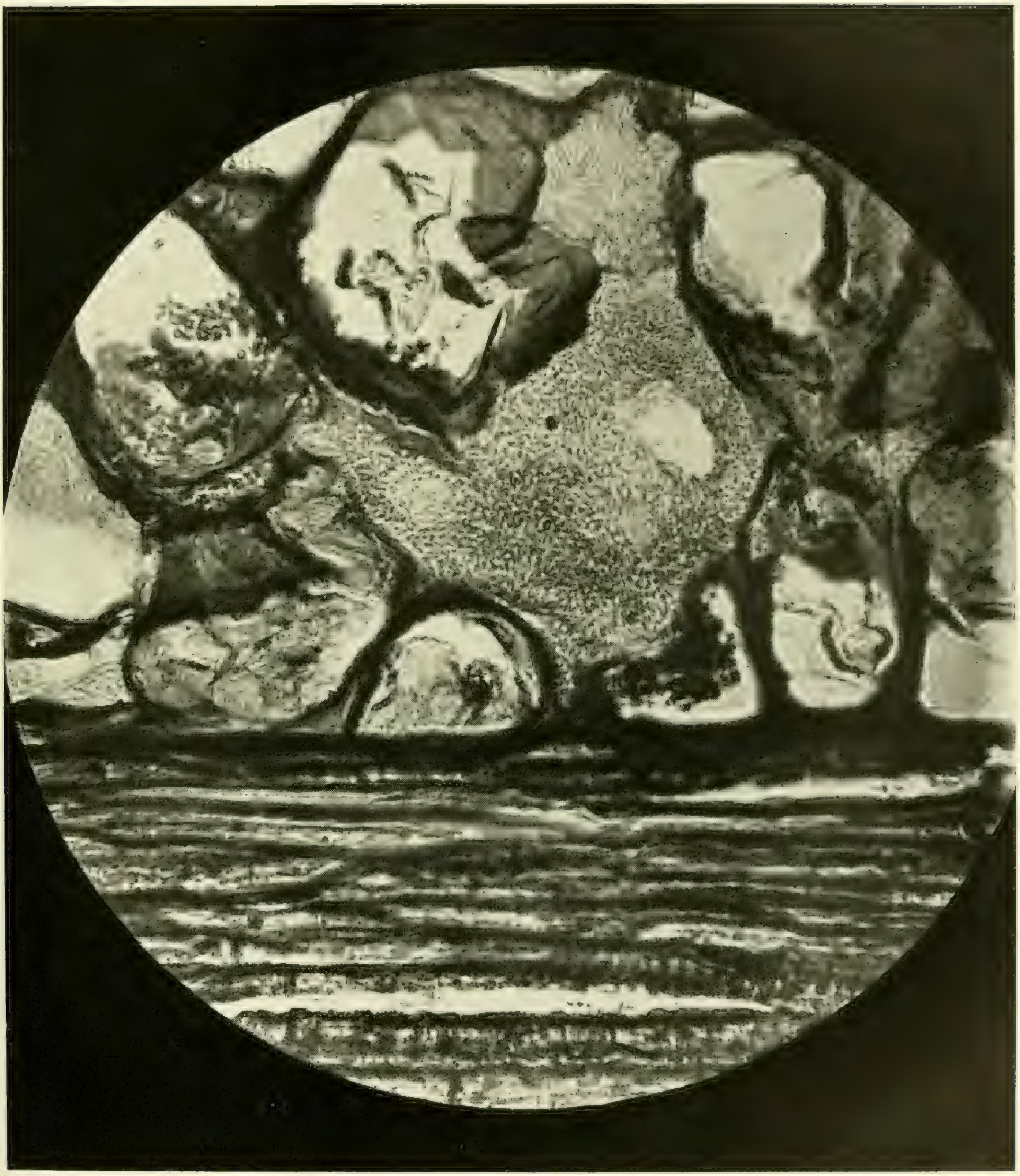


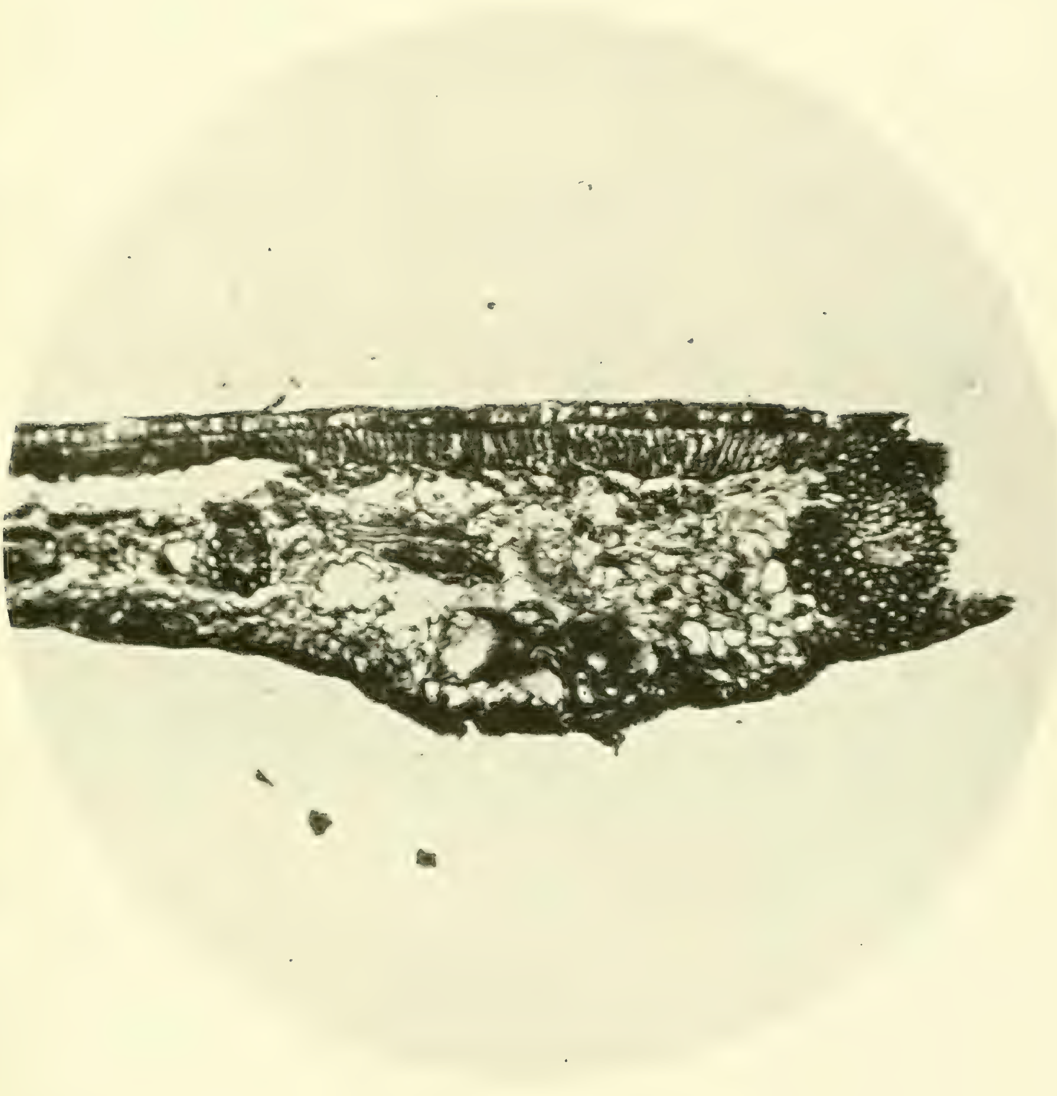












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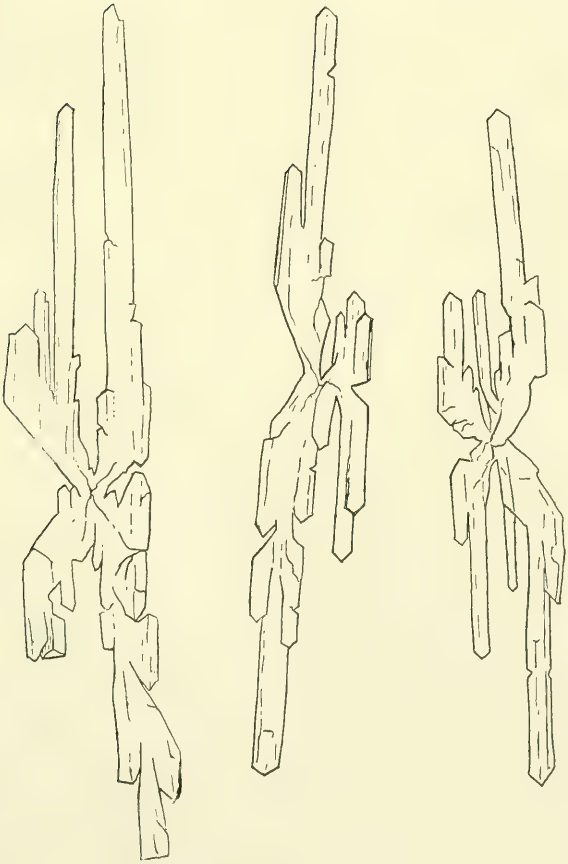

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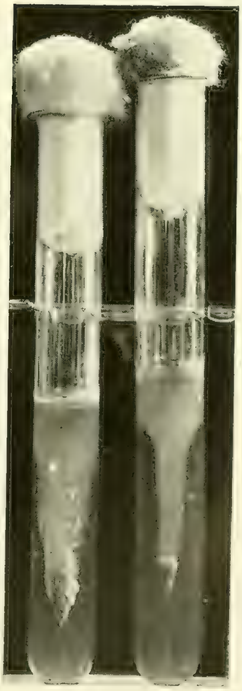

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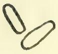
PLATE XII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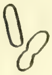
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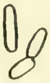
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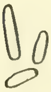
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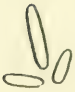
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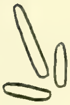
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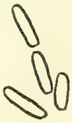
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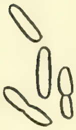
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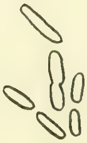
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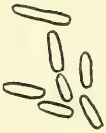
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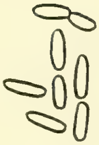
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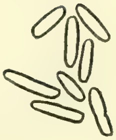
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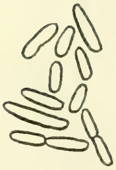
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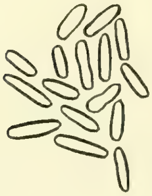
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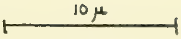

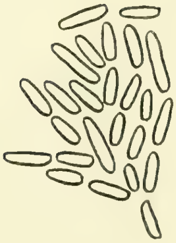
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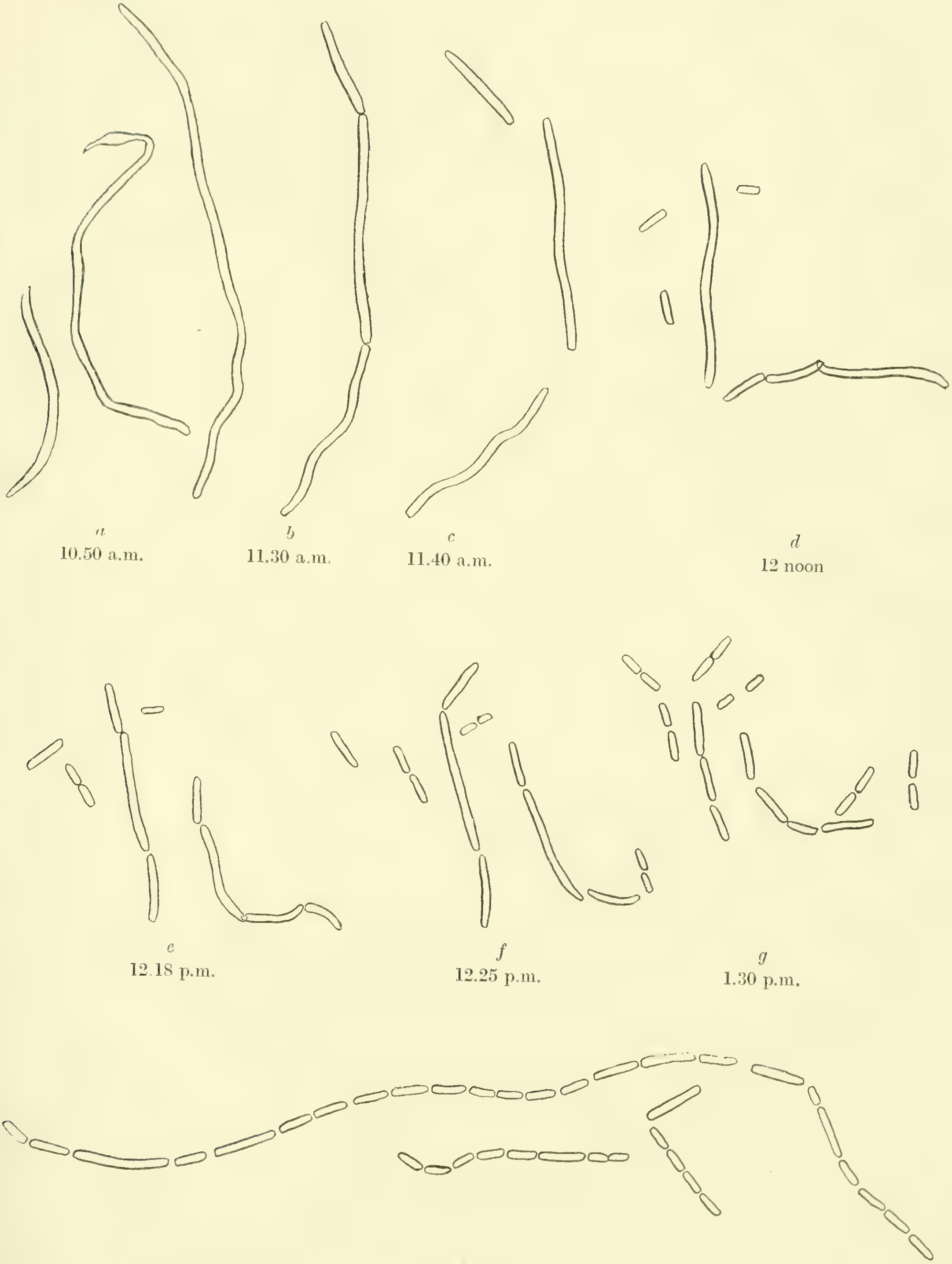
PLATE XIII



a 10.50 a.m.

b 11.30 a.m.

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d 12 noon

e 12.18 p.m.

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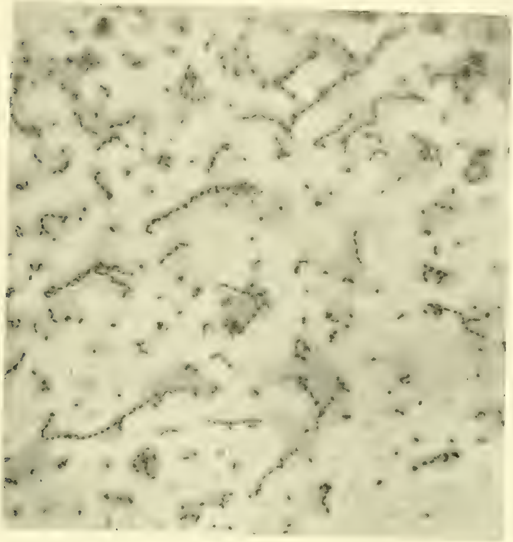

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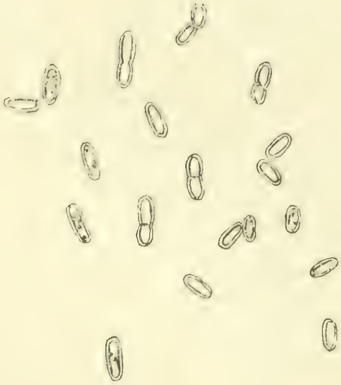

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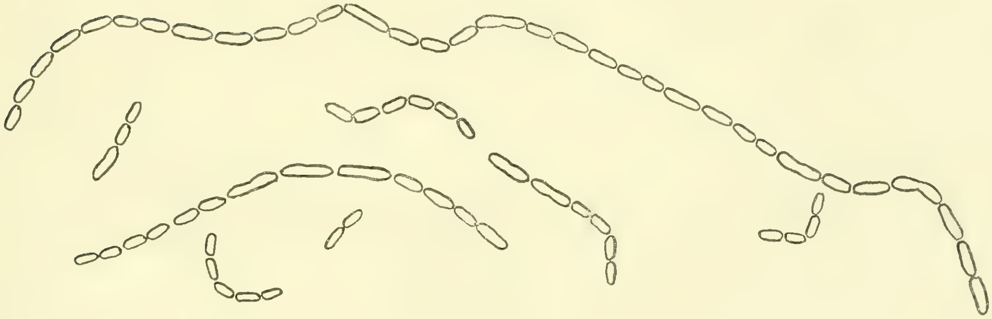

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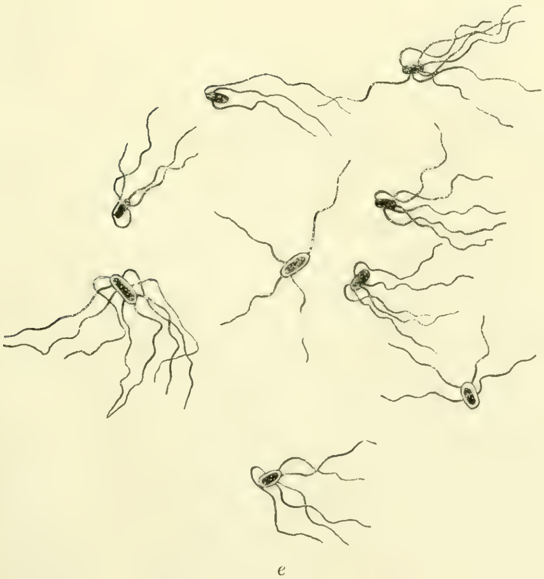

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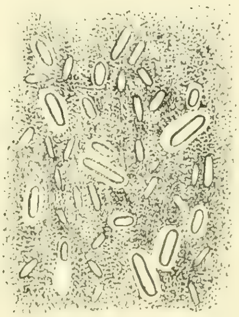


d

Plate V. Mangoes in a more advanced stage of the disease; deep longitudinal cracks have formed.

Plate VI. In this case the whole inflorescence has been destroyed; the one remaining fruit fell off as the photograph was taken.

Plate VII. Photomicrograph of section through fruit, showing an early stage of the disease. The dark area consists of crushed, discoloured cells, with masses of bacilli in the intercellular spaces. (Photographed with Zeiss' achromatic objective D, and compensating ocular No. 6.)

Plate VIII. Photomicrograph of section through fruit in a more advanced stage of the disease. The superficial cells are crushed and dead; there are very few bacilli in this region. They are very numerous in the more deeply seated tissues, a small fibro-vascular bundle at the foot of the plate is surrounded by them, but untouched. Same magnification as Plate VII.

Plate IX. Detail from Plate VIII., in the neighbourhood of the fibro-vascular bundle. The bacilli can be clearly seen in the intercellular spaces, wedging the cells apart. (Zeiss oil imm.; obj. 2 mm., 1·4 app.; comp. ocular No. 6.)

Plate X. Photomicrograph of section through diseased spot on leaf. The increase of thickness due to the swelling of the disorganised cells is very evident; two small vascular bundles in the field have not been attacked. The organism has invaded the tissues from the underside of the leaf, the palisade cells still being intact. There are no stomata in the epidermis of the upper side of the leaf. (Same magnification as Plate VII.)

Plate XI. (*a*) Plate culture of *Bacillus mangiferae* photographed after five days at 25° C.

(*b*) Crystals from plate culture in +15 nutrient agar, 10 days old. Photographed from a drawing made with camera lucida, and Zeiss achrom. obj. AA., ocular No. 1.

(*c*) Stab cultures in nutrient gelatine photographed after 20 days at 20° C.

(*d*) The same culture seven days later.

Plate XII. *a—o*. Development of a small colony as observed on agar hanging block. For fuller explanation see text. (Zeiss 1/12 obj. oil imm. and No. 12 compensating ocular.)

Plate XIII. *a—h*. Stages in the segmentation of long threads developed in nutrient broth containing 8·5 NaCl. Full explanation in text.

Plate XIV. (*a*) Photomicrograph of chains formed in pellicle on nutrient broth, eight days at 30° C., stained with MacConkey's capsule stain.

(*b*) From same preparation as above, drawn with camera lucida, Zeiss oil imm. obj. 1/12 and compensating ocular No. 12.

(*c*) Bacilli from 2 % dextrose broth, three days at 30° C. (Paraboloid condenser, Zeiss apochrom. obj. 3 mm., apert. 0·95, compensating ocular No. 18.)

(*d*) Capsuled rods from ring above nutrient broth, 15 days at 30° C., MacConkey's capsule stain. Zeiss oil imm. obj. 1/12, comp. ocular No. 12.

(*e*) *Bacillus mangiferae* with flagella, from culture on nutrient agar, 20 hours at 25° C. Stained by Ellis' modification of Loeffler's method. Same magnification as (*d*).

[Note by Editor. It has been necessary to reduce the size of some of the figures; for which allowance must be made in the estimate of magnification.

Plates VII, VIII, IX and X have each been reduced by one-eighth.

On Plate XI, figs. *c* and *d* have been reduced by one-eighth.

Plate XIII, all figures reduced by one-sixth.

Plate XIV, figs. *c* and *e* reduced by one-fifth.]

Botanical Laboratories of the Union of South Africa, Pretoria.

TWO SCOLIID PARASITES ON SCARABAEID LARVAE IN BARBADOS.

By W. NOWELL, A.R.C.S.

(*Imperial Department of Agriculture for the West Indies.*)

(With Plate XV and 1 Text-figure.)

As introduction a word should be said regarding the incidence of the rainfall in Barbados and the agricultural operations determined by it, since these have a bearing on the life of the insects to be considered. There is no definite separation into dry and wet seasons, but generally speaking the first five months of the year are characterised by drought, broken now and then by more or less heavy showers; from late May or early June onwards these are supplemented by the occurrence of very occasional falls of two or three inches of rain, producing what may by contrast be called the rainy season. The canes are planted towards the end of this season, about December, and are reaped in March and April, some sixteen months later. After the reaping season, as showers permit, various other crops—among them sorghum and maize—are planted, and these have often accomplished a fair amount of growth by the time that the heavier rains bring in the period of greatest insect activity.

Two beetles of the family Scarabaeidae occur in Barbados. *Ligyrus tumulosis* Burm., a Dynastid, is universally distributed and very abundant. Its larvae live in soil rich in decaying vegetable matter. They occur in very large numbers in pen manure and are to be found at the base of the heaps of dead sugar-cane leaves or cane stumps which are made up at the end of the reaping season. They are not known to attack living plants. The beetle has no definite seasonal distribution, but the adults are capable of remaining for a considerable time in the soil, from which they emerge in large numbers after rain.

Phytalus smithi Arrow, a Melolonthid, though common, is much less noticeable owing to the fact that the adults are very rarely attracted to light. They emerge in large numbers after the May rains and may be found feeding at night on the foliage of various field and garden plants. At other seasons of the year they are very seldom seen, though there is evidence that they occur in small numbers. The larvae attack the roots of many plants, and in gardens are very destructive to rose bushes, but it is as pests of sugar-cane that they are of special importance. Their occurrence in destructive numbers in the Barbados cane-fields is rare and seems confined to small and shifting areas, but in Mauritius, into which island it seems highly probable that they were introduced in sugar-cane stools from Barbados, great and continuous damage is being caused over an ever-widening area.

While investigating root grubs of sugar-cane at Spencers Plantation towards the end of the year 1911 the writer noticed in the soil beneath and about the roots of sugar-cane small numbers of brown cocoons. These proved to contain pupae of a black Scoliid wasp, which was bred out. When forwarded later through Mr G. A. K. Marshall, then Secretary of the Entomological Research Committee, it was identified at the British Museum as *Tiphia parallela* Smith. The specimens then in the British Museum collection, which were females only, had been obtained from Brazil.

Both sexes are shining black in colour, with greyish pubescence on the legs and grey fringes at the joints of the abdomen. The wings are lightly tinted with brown. There is very considerable variation in size among the females, the range in length (excluding the antennae) in a representative collection being from 15 mm. to 9 mm. The males range from 9 to 6·5 mm., excluding antennae and anal spine. The males are readily distinguished by the presence of this spine, which projects from the tip of the abdomen; its rigidity and stoutness sufficiently prevent confusion with the sting of the female, and it has a marked upward curve.

By examination of the remains adhering to the cocoons the writer was able to determine that the host was the larva of Phytalus.

After rain in early June the adult wasps, male and female, were found in large numbers in the same locality feeding on the honeydew on aphis-infested sorghum. They were observed in greatly fluctuating numbers until about the end of September, and it was noticed that towards the end of the period there was a large preponderance of males. In 1913 large numbers again occurred in June, in practically the same place. That their emergence is not confined to the wetter months is proved by the finding at all periods of the year of newly parasitized larvae. Possibly there is some check to emergence during dry weather, though experiments in the insectary have not supported the supposition. Of 21 laboratory-reared examples, each of which had pupated in earth in a separate Petri dish, 11 which were kept air-dry averaged 35 days, and 10 which were kept moist averaged 36 days in the cocoon. The periods ranged between 32 and 40 days, with one exception in each case: one of those kept dry emerged in 47 days, one of those kept moist in 45 days. More observations are needed, but partly at any rate the finding of large numbers in the wetter months must be ascribed to the opportunities for collection afforded by the attraction of the honeydew. No instance of their visiting flowers has been observed. Of the distribution of Tiphia about the island little is known, and that mainly of a negative character. An outbreak of Phytalus, small in area but rather severe, in a cane-field at Waterford Plantation, 10 miles from Spencers, was examined in May 1913, and amongst a large number of cane stools dug up only one Tiphia cocoon was found. The Manager of Spencers Plantation states that a similar outbreak of Phytalus took place some three years back in the fields where Tiphia is now so common. It would appear that we have quite sufficient room in the small area of Barbados for the typical sequence of a local outbreak of a pest followed by the migration and ultimate preponderance of its parasite.

Under these circumstances the percentage of parasitism depends upon the stage which has been reached in the local cycle. In the well-developed example at Spencers the number of cocoons and parasitized grubs found during extensive digging amounted to some thirty per cent. of the total number of grubs and cocoons found. There being several generations of Tiphia to each generation of Phytalus; this figure must be much below the actual one, since no account was taken of the large number of empty cocoons representing recent generations of Tiphia, nor does it include the number of grubs which would still have been attacked before pupating.

Information as to the life history of the parasite was first obtained from discoveries of early stages in the field. Later the stocking of Wardian cases for an attempt to introduce it into Mauritius afforded further opportunities, especially for watching the behaviour of the adult wasps, while it has recently been found possible to carry through successive generations in the insectary.

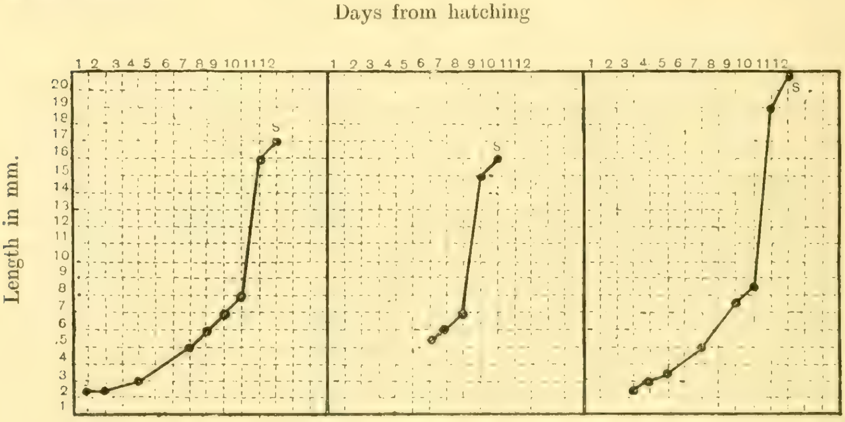
The largest number of eggs actually obtained from one female in captivity is six, but an examination of the ovary tubes seems to indicate an egg capacity of at least seventy.

The laying female may be seen in the field running quickly over the surface of the soil, her antennae vibrating rapidly all the while. As she obtains the required indication she commences to burrow, shuffling forward with her legs and appearing to use her head to separate the particles of soil.

Tiphia has never been induced by the writer to take any notice of a grub on the surface, but the method of attack was seen in the case of a half-buried grub and closely resembled that of *Campsomeris dorsata*, to be described in detail later. Neither Tiphia nor Campsomeris will lay on a grub close to the surface, but after stinging it into quiescence each proceeds to burrow under it and drag it down. The Tiphia mentioned above took several minutes in getting her prey out of sight, during which it recovered activity after the first sting and received a second.

The egg is laid transversely in a fold of the dorsum of the thorax, and is firmly agglutinated throughout its length by a cement which is colourless at first but eventually becomes dark brown. The egg measures a little over a millimetre in length. It is quite white when first laid but soon darkens slightly. In no case under natural conditions has more than one perfect egg been found on one grub. Occasionally a grub has been found in the field with an egg or larva upon it and in addition to this a brown elongated spot has occurred, apparently marking the position of a previous egg. In the insectary, where the operations of several females were confined to a limited number of grubs in the same flower-pot, such cases were very common and the remains of two eggs in addition to the living one were sometimes found on the same grub. It appears somewhat probable that when a wasp about to deposit her egg finds one already there she destroys it.

The egg stage occupies 5–6 days. At the end of this time the membrane splits transversely close to one end, which then forms a cap hinged on its dorsal margin. From the opening thus made the head and one or two additional segments of the larva are extruded. While thus situated the larva punctures the skin of the grub with its mandibles. The perforation is very minute and is very little enlarged during the remainder of the life of the host. The head of the larva is closely applied to the hole, but is not pushed into it and is quite free to move. Whether in feeding there is an actual suction by the larva, or whether the fluid simply wells out from the wound and is imbibed from the surface, was not determined. Continuous movements resembling peristalsis may be seen within the body of the parasite. On the day following that of hatching the egg membrane splits dorsally along its length, and the back of the larva is revealed, but the larva still firmly adheres by its ventral surface to the egg membrane below, thus retaining its attachment to the host. The successive moults are accomplished in a similar way: the skin splits along the back and gradually recedes down the sides of the larva as the latter increases in circumference. The number of moults was not determined with absolute certainty; in addition to that which occurs on passing into the pupal stage two were observed during the time that the larvae are attached to the host, occurring in normal examples 3–4 days and 7–8 days after hatching.



S indicates spinning
Growth in length of three Tiphia larvae

The increase in length of three typical examples is shown in the subjoined diagram. Until about the tenth day from hatching growth is steady and regular, this being the period during which the host remains alive, or at least, retains its normal shape and turgidity. Up to the last day or two of this period it is quite active, burrowing through the soil and in some cases feeding. On the last day it is without movement, and the large anal segment has usually become translucent. Until this stage is reached the parasite has retained its original attachment, becoming considerably arched as it increases in length, and hanging to one side across the back of the host like a firmly stuffed pillow. It now pushes out its long tapering neck, hitherto concealed, and attacks the actual tissues. It usually begins behind the head, eating a large hole into the thoracic segments of its now collapsed and detached victim. The whole body is cleaned out in a few hours, only the shrivelled skin, with the feet and the hard brown integument of the head, remaining. The increase in size during this final stage of feeding is remarkable, the linear proportions being more than doubled.

The larva proceeds to spin up *in situ*, the long neck facilitating the attachment of the thread to different points in the wall of the cavity previously occupied by the host. A very loose fluffy outer covering is first formed, and within this a firm-walled, extremely tough, elongate-ovate inner envelope. The whole cocoon is whitish at first, but darkens to a tawny brown.

The process of spinning, so far as it can be externally watched, occupies about 24 hours.

The time spent in the cocoon, as observed in the insectary, is usually from 32–40 days. Only in three instances in the writer's experience has this been exceeded, in which cases periods of 45, 47, and 56 days were reached. Excluding these, the average of 22 carefully recorded examples was 35 days.

The wasp emerges by biting an irregular hole through the cocoon near one end, generally the larger one. It may be heard rasping away the envelope at that point with its mandibles an hour or so before its head appears.

Both sexes take food, if it is available, immediately after emergence. In captivity cane syrup, sugar and water, and honey are readily accepted.

Copulation has been observed both in the field and in captivity. If, however, a virgin female is confined over soil containing Phytalus grubs she will proceed to lay fertile eggs. The experiments which have established this were carried out with females reared separately in small Petri dishes. Phytalus grubs, examined under a dissecting microscope to make sure that they were free from eggs, were buried in soil mixed with a few fibrous roots in large flower-pots, made with a bulging rim to fit closely round the base of large lantern glasses. The top of each glass was covered with tissue paper held tight by a cardboard ring. A hole was punched through the paper with a pencil, the female (slightly chloroformed) introduced, and the hole closed with gummed paper. Difficulty in obtaining grubs limited the experiment, but some fifteen eggs were obtained from five females. All hatched and produced normal larvae. Owing to external circumstances only three adults were secured from these eggs, all males.

In view of experiments in introducing this or other species of Tiphia for the control of Melolonthid grubs in other West Indian Islands and perhaps elsewhere it may be of value to give the results of the writer's experience in Barbados. The most obvious procedure is to dig up and transmit the cocoons, but success has not ensued from the practice of this method. Large numbers of cocoons have been dug out under the writer's personal supervision. They have been exposed as little and handled as carefully as possible, kept on or in soil or in various receptacles, in different degrees of moisture. Always the number emerging has been disappointingly small, with no observed indication of the cause of failure. Under each condition tried, except when the cocoons were completely buried, a small percentage emerged, while the rest of the cocoons when opened showed larvae, pupae or imagos dead and dry. Buried cocoons failed altogether. It seems most probable that mechanical injury is inflicted in the disturbance involved in digging up and transporting the cocoons. The tenderness of Lepidoptera at the same stage will occur as an analogy to the entomologist. It scarcely needs to be added that shipment of such cocoons, in cool storage or by parcel post, has not been attended with success.

The method by which living material was eventually landed in Mauritius was as follows: Wardian cases with a sugar-cane stool established in each were stocked with healthy Phytalus grubs, and Tiphia imagos, male and female, were introduced and fed with syrup and water. Many of the grubs were later found to be parasitized, others had completed their development and commenced a new generation of Phytalus in the cases. Fresh grubs were introduced as they were obtained up to the date of shipment. The only information I have received was that from this material wasps were obtained in Mauritius which laid on Phytalus grubs in the insectary there. For introductions where the time taken in transit does not exceed the length of the life cycle of Tiphia it would be sufficient to have a number of boxes containing grubs in soil, to cover each for a few days with a cage of mosquito netting within which Tiphia imagos could be confined, and then to remove the cage and transmit simply the boxes with the parasitized grubs. Probably success might be attained in sending cocoons, provided these had been formed in the insectary in small boxes of soil, matchboxes say, so that they could be sent with a minimum of disturbance. In any case, unless the indication that the parthenogenetically produced wasps are males should fail to be confirmed by further experiments, it will be necessary to send a sufficient amount of parasite material to give a reasonable chance of males and females emerging about the same time.

The second Scoliid to be described, *Campsomeris* (*Dielis*) *dorsata* (Fab.), is found in Barbados throughout the year. The wasps occur very abundantly on flowers at frequent and apparently irregular intervals, sometimes both sexes together, sometimes with one or the other greatly preponderating. Their visits to flowers cease shortly after mid-day. The males have a curious habit, so far observed only in the afternoon, of collecting in large numbers on any convenient piece of vegetation, such as a grass stem or a yam vine, forming an assembly like a swarm of bees. The writer is quite at a loss to account for this habit, which occurs in several other Fossorial wasps met with in the West Indies.

The sexes are very different in appearance. The male is slender, 12–17 mm. in length. The dorsal surface of the abdomen is striped laterally with yellow and black, the ventral surface is blue-black; the second (apparent first) abdominal segment is considerably smaller in circumference than the remainder. A narrow yellow band bordered with black makes an almost complete circle on the dorsum of the thorax at the level of the wing insertions and is repeated behind. The antennae are cylindrical, about 8 mm. long.

The female is stouter and has a length of 14–24 mm. The colour is a uniform shining black with the exception of a brick-red blotch which covers the dorsal aspect of segments three and four of the abdomen, divided only by a black line at their junction. The wings are of a deep metallic blue. The antennae are about 4·5 mm. long. There is considerable greyish pubescence on the thorax, legs, neck and waist, and on the front of the head.

The notable range in length observable in each sex of both Tiphia and Campsomeris is not a regular variation about a central mean. The larger sizes are more abundant. The tendency seems to be towards the attainment of the maximum for the sex, limited by the nutrition available from the single larva of the host. Attention has not been specially directed to the question as to whether there is any tendency to the selection of large grubs by the laying female, but quite small grubs have occasionally been noticed to be parasitized. Observation has seemed to show, quite definitely in the case of Ligyrus, that in a continuous breeding place small larvae are much less common at any one time than those approaching full size, due presumably to the earlier stages of growth being passed over more rapidly, which, granted the influence of nutrition, would sufficiently account for the relative infrequency of the smaller sizes of the parasite. The whole question is perhaps worthy of direct investigation.

The life history of this wasp was worked out during 1912 at Spencers Plantation. Since it had not at that time been found by the writer in connection with Phytalus, the probabilities pointed to Ligyrus as its host, and a search revealed, associated with Ligyrus in pen manure, an abundance of parasitic larvae and cocoons which turned out to be those of Campsomeris.

When pen manure, containing large numbers of Ligyrus grubs, was being put out in the fields and buried alongside the young clumps of sugar-cane in February 1913, large numbers of the wasps, male and female, were seen in the same fields; the males were darting rapidly about just above the ground, the females were flying from one cane clump to another and alighting to search the surface of the soil over each place where the manure was buried. Many were seen to burrow rapidly out of sight in such situations. They remained below for very various lengths of time, and in most cases digging at the spot where the wasps had burrowed revealed, at a depth of from six inches to a foot, a larva of Ligyrus alive but stupefied and with an egg upon it.

A healthy larva of Ligyrus was dug up and placed on the surface of the soil near to a hunting female Campsomeris. The latter quickly took notice of it, and a struggle ensued between the two which lasted about five minutes. The wasp, before she would use her sting, manoeuvred for a hold which would enable her to plant it in the right place, which proved to be a spot between the legs of the larva, as near as could be made out between the third pair. Three times when the wasp faced the grub for this purpose some part of her head or its appendages was seized between the mandibles of the grub. When she finally succeeded in inserting her sting the grub immediately relaxed and became quiet. The wasp then proceeded to burrow under it, and after loosening the soil, she pulled it under. This was about 5.15 in the evening.

The place was marked and visited early next morning, when the grub was found buried ten inches deep, with an egg upon it. The wasp, in an exhausted condition, was turned up at the same time. There was no manure within several feet of this place, so that it is unlikely that a second grub was mistaken for the first.

The egg of Campsomeris is attached by one end about the middle of the ventral surface of the grub. It is easily separated and the resulting larva is not attached to its host. The difference from Tiphia in these points is no doubt correlated with the fact that the Ligyrus grub does not recover its activity after being stung by Campsomeris, but remains in a quiescent condition, making only slight movements of its legs when disturbed. Since, as will be mentioned later, Phytalus is occasionally successfully parasitized by this wasp, it appears likely that its sting has the same effect on the larva of that insect.

The development of the Campsomeris larva is very similar to that of Tiphia. The history of the specimen above mentioned may be continued as an example. The egg was laid during the night of the 13th or, more probably, early in the morning of the 14th of February. The further observations made are summarised below:

Date	Day	
Feb. 14	1	Grub with the newly deposited egg placed in insectary.
,, 15	2	Not hatched at 1 p.m.
,, 16	3	No observation.
,, 17	4	Egg found to be hatched when examined at 10 a.m.
,, 21	8	Parasite well grown; host still shows faint movements.
,, 22	9	Parasite growing fast; host doubtfully alive.
,, 24	11	Host quite dead, skin half empty; parasite more than doubled in length since last observation.
,, 25	12	Skin empty except for earthy matter in alimentary canal. Parasite disengaged itself during day and began to spin up.
,, 26	13	Cocoon getting well into shape at 9 a.m.

The actual date of emergence is not known. The cocoon was found empty on April 15th, the wasp having escaped unnoticed.

The duration of the egg stage was thus 3–4 days; the host remained alive 6–7 days after the parasite hatched; spinning began on the 9th day after hatching. The duration of the cocoon stage was not more than 48 days, probably some days less.

The cocoon of Campsomeris is easily distinguishable from that of Tiphia. The shape is cylindrical-oval. The outer envelope is not loose and fluffy as in Tiphia, but has a consistency like that of loose-textured tissue paper. It encloses the inner envelope somewhat slackly. The method of emergence is also different: the wasp cuts round the cocoon near one end and pushes to one side the neat cap thus formed.

Campsomeris is by far the most common of the flower-visiting Hymenoptera in Barbados, but although it is very plentiful the percentage of Ligyrus parasitized at Spencers has not been found to be large in the situations explored. Pond mud, of close texture and very firm, is largely used in making up the pen manure, and seems to afford protection to large numbers of the grubs. As described above, when this material is more or less broken up by distribution in the fields, the wasps collect about it in a way that is not seen before it is disturbed.

In digging up cane stools in the fields an imago or a cocoon of Campsomeris was occasionally met with in the situations usually occupied by Tiphia, and in the extensive digging operations connected with the shipments to Mauritius it was definitely established by the examination of the skins attached to these occasional cocoons that Campsomeris is to a small extent parasitic on Phytalus[1]. The ratio of this parasitism to that due to Tiphia at the same time and place was calculated to be about one hundredth. The writer has never found Tiphia on Ligyrus.

A Rhipiphorid beetle, identified through the Imperial Bureau of Entomology as *Macrosiagon octomaculatus* Gerst., has several times been found to emerge from cocoons of Campsomeris, and has been taken on flowers of *Antigonon leptopus*, which are much frequented by the imagos of that wasp. No direct information has yet been obtained as to its method of parasitism, but it is probably that common to the order, the eggs being laid on or near flowers, the young larva attaching itself to a wasp visiting the flowers, and transferring itself when the egg of the wasp is laid. Numbers of a triungulin whose relations have not been ascertained were recently found on several Ligyrus larvae which bore eggs of Campsomeris.

So far observations have shown that Tiphia, which does not visit flowers, completely escapes this parasite.

The investigations described in this paper were carried out in connection with the Barbados Department of Agriculture, and are published by kind permission of Mr J. R. Bovell, I.S.O., F.L.S., F.C.S., Superintendent of Agriculture. The somewhat ragged state in which certain matters have been left is due to my transfer from that Department and the assumption of duties of a different nature. I am specially indebted to Mr A. A. Evelyn, of Spencers Plantation, without whose interest and co-operation the field work would have been impossible, and my thanks are due to my present colleague, Mr H. A. Ballou, M.Sc.,

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[1] An earlier record of the parasitism of Campsomeris on Phytalus is contained in a letter from Mr G. A. K. Marshall to the Superintendent of Agriculture in Barbardos, mentioning the finding of one example at Spencers, in February 1912. This is the occurrence referred to in the Report of the Bureau of Entomology, 1914.

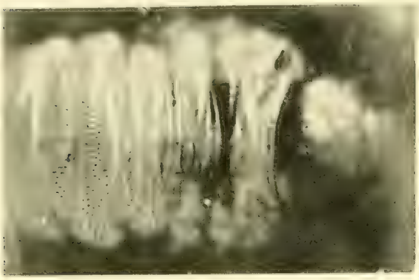


Fig. 1

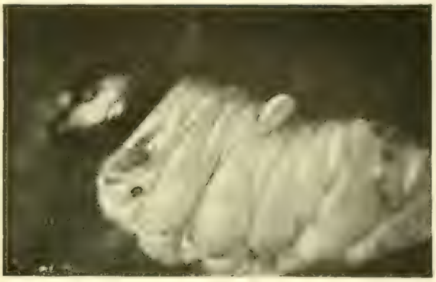


Fig. 2

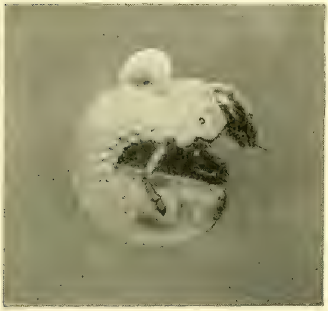


Fig. 3

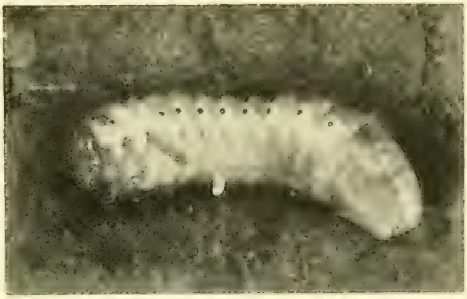


Fig. 4

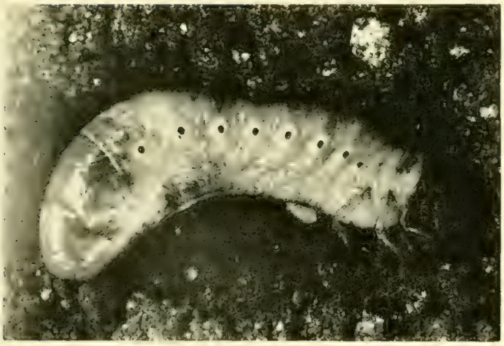


Fig. 5

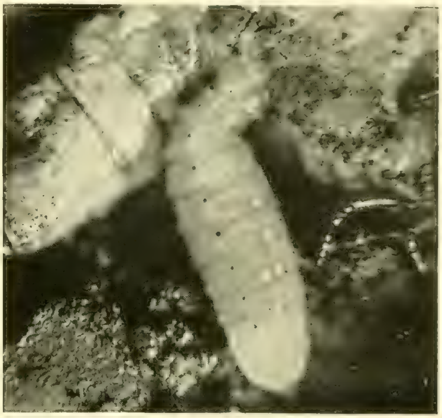


Fig. 6

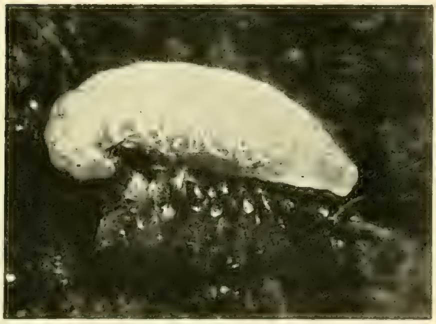


Fig 7

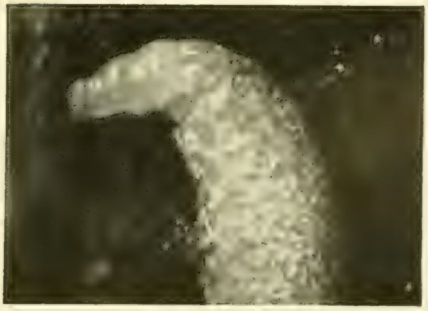


Fig 8

Entomologist to the Imperial Department of Agriculture, with whom I have discussed various points arising in the course of the research and in the preparation of this paper.

EXPLANATION OF PLATE.

Fig. 1. *Tiphia parallela*: egg on Phytalus; × 4¼.
Fig. 2. ,, larva three days old; × 4¼.
Fig. 3. ,, larva eight to nine (?) days old; × 1½.
Fig. 4. *Campsomeris dorsata*: egg on Ligyrus.
Fig. 5. ,, ,, larva one day old.
Fig. 6. ,, ,, larva beginning to attack tissues of host; × 4¼.
Fig. 7. ,, ,, larva at end of feeding period; × 3.
Fig. 8. ,, ,, larva beginning to spin up; × 4¼.

(Fig. 3 from a photograph by H. A. Ballou; remainder by the author.)

PINK DISEASE OF PLANTATION RUBBER.

By F. T. BROOKS, M.A.

(*Senior Demonstrator of Botany, Cambridge University, and formerly Government Mycologist, Federated Malay States*),

and A. SHARPLES, A.R.C.S.

(*Assistant Mycologist, Federated Malay States*).

(With Plates XVI and XVII and 11 Text-figures.)

Introduction.

There has been a considerable development of Pink Disease in Malayan rubber estates since 1912 and at the present time it is the disease which requires the greatest amount of attention in many districts. We have seen several estates in which 10 per cent. of the trees were affected and in a few neglected plantations in parts of the country where the disease was bad no less than 25 per cent. were attacked. During 1914 an opportunity was given us of investigating this disease in detail especially as regards plantation rubber and the present paper embodies the chief results of this investigation. Other information about the disease, which is of interest only to planters, is included by us (4) in Bulletin 21 of the Department of Agriculture, Federated Malay States.

Distribution and Hosts.

Pink Disease is caused by a fungus which was named *Corticium javanicum* in 1901 by Zimmermann (17) who investigated it in Java with special reference to coffee, but Petch (9) points out that the same fungus had been named *Corticium salmonicolor* by Berkeley and Broome many years before from material obtained in Ceylon, hence the latter name has the right of priority.

In 1897 Ridley (11) reported the presence of a disease of coffee in Selangor caused by a fungus with pink spore masses, which was named

Necator decretus by Massee (8), and in 1901 Zimmermann (17) reported the same fungus on coffee in Java and pointed out that it was generally associated with *Corticium javanicum*, not only on coffee but also on tea and other hosts.

Between 1904 and 1909 the fungus was reported under different names on rubber in North Borneo (12), Malaya (6, 13, 14), Ceylon (9), and Southern India (1). More recently it has been found on rubber in Sumatra (10) and Burmah (4). *Corticium salmonicolor* has been found on other hosts in the Cameroons (5) and in the Caucasus (15). In the West Indies a pink fungus on cacao has been referred to *Corticium lilacofuscum*, which may possibly be identical with *Corticium salmonicolor.*

In 1912 Rant (10) published an account of an investigation of *Corticium salmonicolor* with special reference to cinchona, the most important result of which was to establish the identity of the *Corticium* and *Necator decretus*, these being shown to be two stages of one and the same fungus.

Corticium salmonicolor is an omnivorous fungus and Rant mentions that it has been found on no less than 141 species of plants belonging to 104 genera and many different families. The disease has been found on Gymnosperms as well as on Dicotyledons, but no record has yet been made of it on Monocotyledons. Rant (10) states he has seen the fungus growing on the epiphytic fern *Drymoglossum heterophyllum* without apparently causing harm.

In Malaya, *Corticium salmonicolor* has been found recently on Para rubber, cocoa, *Coffea robusta*, *Gardenia sp.*, *Hibiscus sp.*, camphor (*Cinnamomum camphora*), *Cassia sp.*, horse mango (*Mangifera foetida*), *Lansium domesticum*, lime, durian (*Durio zibethinus*), jak (*Artocarpus integrifolia*), *Averrhoa sp.*, mango (*Mangifera indica*), *Tephrosia Hookeriana*, *Indigofera arrecta*, and *Clithoria cajanifolia.* The number of fruit trees in this list is noteworthy, but the fungus is not often found on them.

In other tropical countries the fungus is of economic importance on other plants besides rubber. In Java, coffee and cinchona are seriously affected by it and in Ceylon the fungus causes a serious disease of tea.

Corticium salmonicolor is probably native in most of the countries in which it has been recorded. Many of the plants mentioned by Rant (10) as having been affected by it are native to Java and some of the plants on which the fungus has been found in Malaya are also indigenous. Anstead (1) states that the fungus is present on jungle trees in the neighbourhood of rubber estates in Southern India. The fungus has probably spread from native hosts to plants that have been introduced, such as rubber, coffee, tea, and cinchona. There is one doubtful record of it on a jungle tree in Malaya, but one cannot say at present whether it occurs to any appreciable extent in the forests. Unfortunately *Corticium salmonicolor* has shown a considerable liking for rubber trees, and as far as Malaya is concerned *Hevea brasiliensis* is by far the commonest host for the disease. The disease is much more prevalent in some parts of the country than in others and there are some large areas where the disease has not yet been found. The disease is most abundant at present in the districts of heaviest rainfall and where large tracts of jungle remain.

Field Observations.

Pink Disease attacks rubber trees of various ages though it is not often seen on trees less than two years old. An attack often begins in a fork of a tree on account of the accumulation of water there, but sometimes the disease affects a branch in the middle and it has been seen occasionally to attack the main stem. The disease develops most rapidly during periods of heavy rain. In dry weather obvious signs of the fungus frequently disappear to appear again when the rains come.

The manifestations of Pink Disease on rubber trees are extremely variable. The disease is so called because the fungus often causes a pink incrustation on the branches or main stem, which is more specially developed on the under or shady side (shown on the right-hand side of Plate XVI, fig. 2). In this condition the disease is very striking and cannot be mistaken. The incrustation cracks irregularly after a time and the bright pink colour rapidly fades to a dingy white. There are, however, at least three other forms in which the fungus appears on rubber trees:

(*a*) Pink Disease frequently assumes the form of white or pale pink pustules arranged more or less in lines parallel with the branches. This is the "Höckerchen" form of Rant (10).

(*b*) At other times part of the fungus on the exterior consists of white or pale pink strands of a cobweb-like texture which run irregularly downwards over the surface, the strands being sometimes so delicate as to be overlooked (cf. Plate XVI, fig. 1). This is the "Spinnegewebe" form of Rant (10) and is usually the first stage to appear on an affected tree.

(*c*) Finally there is the *Necator* stage which was formerly looked upon as a separate fungus, *Necator decretus*, Mass., but is now known to be a stage in the life-history of Pink Disease. Pustules of *Necator* are seen on the left-hand side of Plate XVI, fig. 2. The fungus in this condition consists of orange-red (not pink) pustules about one-eighth inch in diameter, each pustule being a mass of spores which serve to propagate the disease. In our experience the *Necator* stage has been confined to the side of the branch which is exposed to the brighter light. The *Necator* stage has always been found by us to be associated with other forms of Pink Disease, and so intimate is the connection between it and the other forms that it is difficult to understand the doubt that formerly existed as to the identity of *Necator decretus* and *Corticium salmonicolor.*

Spores of the fungus germinate on healthy bark especially where there is an accumulation of moisture. The mycelium which develops is entirely superficial at first, but after a time it penetrates the bark. When the mycelium reaches the laticiferous tissues, exudation of latex frequently begins and this runs down the bark and becomes blackened as time goes on. The weeping of latex from branches is often an indication of the presence of Pink Disease when from ground level no other sign of the disease can be seen. Once the mycelium has penetrated the bark it spreads upwards and downwards over and through the bark causing it to rot. The mycelium spreads more rapidly over the bark than through it. The fungus sometimes advances into the wood, this happening more frequently in small branches than in large ones. If the fungus spreads in the wood, the water supply becomes checked and the foliage of the affected branch turns brown and dies. On some undulating estates planters discover those trees which are affected by Pink Disease by observing from a hill the branches which are affected in this manner. Further information concerning the presence and development of the fungus in the wood is given below. Occasionally the fungus spreads downwards so vigorously that the whole of the upper part of the tree dies. In such a case as that represented in Plate XVII, fig. 3, the lower part of the tree sometimes makes an effort to recover by putting out new branches. When large branches are attacked by *Corticium salmonicolor* the progress of the fungus in the bark may be checked by a spell of dry weather and in this case an open, canker-like wound such as is shown in Plate XVII, fig. 4 is often formed as described by Petch (9). The formation of a callus on the margin of the wound tends to repair the injury and occasionally the disease is entirely thrown off, but the fungus sometimes develops again over the bark which began to close the wound. Where the cankered areas have entirely thrown off the disease, the region around them is frequently blackened on account of oxidation of the rubber exuded when the disease was active.

Investigation of Hevea wood affected by Corticium salmonicolor.

Before the appearance of Rant's paper (10), Petch (9) stated that *Corticium salmonicolor* does not enter the wood to any appreciable extent. Rant noticed in cinchona that the fungus invaded the wood and the pith.

Branches of *Hevea brasiliensis* attacked by Pink Disease die in a manner characteristic of those attacked by a fungus which grows vigorously in the wood. Young branches are more often affected in this manner than are old ones. The rapid death of the leaves in such branches is a sure sign that the water supply is checked; this restriction is probably due to the activities of the fungus in the wood. It soon became clear that the fungus entered the wood of such branches, so a detailed investigation of the manner in which it invaded these tissues was made. Wood affected by *Corticium salmonicolor* is only slightly discoloured and differs greatly in this respect from wood permeated by *Diplodia cacaoicola.*

During the early part of 1914 a large branch of a rubber tree attacked by Pink Disease was obtained from an estate in Negri Sembilan in which the transition between healthy and diseased wood could be clearly traced. The branch was covered with the pink incrustation which ran out below into the cobweb-like form of the fungus. The cortical layers were dead and could be easily stripped off exposing the wood beneath.

When split longitudinally, the wood was seen to be sound for about two feet from the top of the branch, but the part below was dry and obviously diseased, except for a length of about nine inches which marked the transition between the healthy and diseased wood. This transition region had a moist, almost transparent, appearance and gradually passed below into the dry, diseased wood and above into the moist, healthy wood.

Diplodia cacaoicola, P. Hennings, is a common wound parasite of *Hevea brasiliensis* and has been found in some branches attacked by Pink Disease. A careful search for *Diplodia cacaoicola* was therefore made before investigating the mode of attack of *Corticium salmonicolor.* No external sign of *Diplodia* was observed and microscopical examination failed to show any of the characteristic dark coloured hyphae of this fungus running through the vessels even in the most badly attacked portions of the wood.

Though *Diplodia cacaoicola* was absent, the wood was permeated with hyaline hyphae. A section of the branch including the transition area and a portion of the dead wood was taken and cut into numbered blocks throughout its length. Razor sections of the wood at different levels were made, the lower portion of the transition area being found most favourable for examination. Transverse sections through this part show the hyphae ramifying through the elements of the wood, being especially prominent in the vessels (Fig. 1). The wood of *Hevea brasiliensis* is mostly composed of fibrous elements together with a comparatively small number of large vessels and narrow medullary rays.

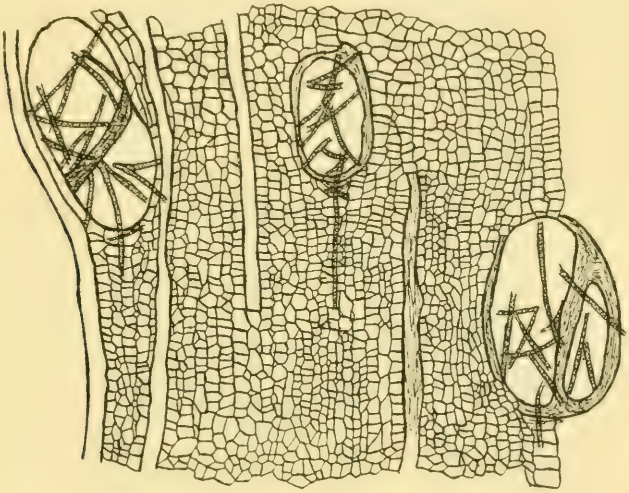


Fig. 1. Transverse section of wood showing hyphae in the vessels. ×40.

A study of longitudinal sections, both radial and tangential, shows the nature of the attack upon the wood. A favourable radial section shows the hyphae passing transversely through the wood along the medullary rays by way of which also the fungus passes from the bark into the wood. The fungus obtains food from materials stored in the ray cells which become filled with septate hyphae (Fig. 2). In radial section the medullary rays appear to be broad bands of infected tissue passing through the wood. At places where the vessels meet the medullary rays, the mycelium travelling in the cells of the rays spreads out and enters the vessels (Fig. 3). All the elements of the wood become permeated with the hyphae which pass readily through the large pits without constriction. The deep medullary rays favour a quick passage transversely while the large pits allow a ready passage for the hyphae among the elements of the wood.

The most characteristic feature in the wood of *Hevea brasiliensis* attacked by Pink Disease is the presence of tyloses in the vessels (Figs. 4 and 5). Every specimen examined showed these bladder-like ingrowths from the living cells bordering the vessels, plugging up the water courses. Specimens of healthy wood, of wood taken below the tapping area, and of wood attacked by *Diplodia cacaoicola* were examined, but in no case was there any indication of tyloses. Thus the formation of tyloses in *Hevea brasiliensis* appears to be a response to the attacks of *Corticium salmonicolor.*

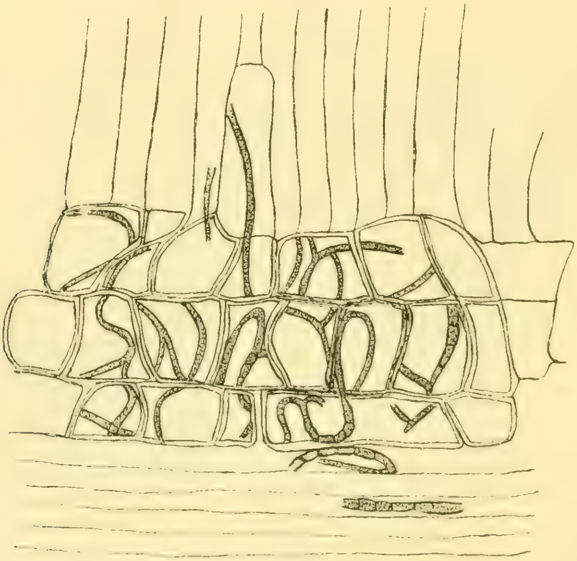


Fig. 2. Section showing hyphae in medullary ray cells. × 160.

The tyloses are of two types, (*a*) those in which the cells retain their thin cellulose walls (Fig. 4), (*b*) those in which the walls become lignified (Fig. 5). Both types are found in the same branch in adjoining vessels though the second type is rarely produced. The tyloses which become lignified lose their protoplasm and appear like a number of small vessels included in a larger one. In the majority of cases only the thin-walled type occurs. Their contents are devoid of food reserves.

The response made by living cells to an injury usually results in an abnormal growth of neighbouring cells. In *Hevea brasiliensis* attacked by Pink Disease an abnormal bladder-like ingrowth of the living cells bordering the longitudinal path of the fungus, *i.e.* the vessels, takes place.

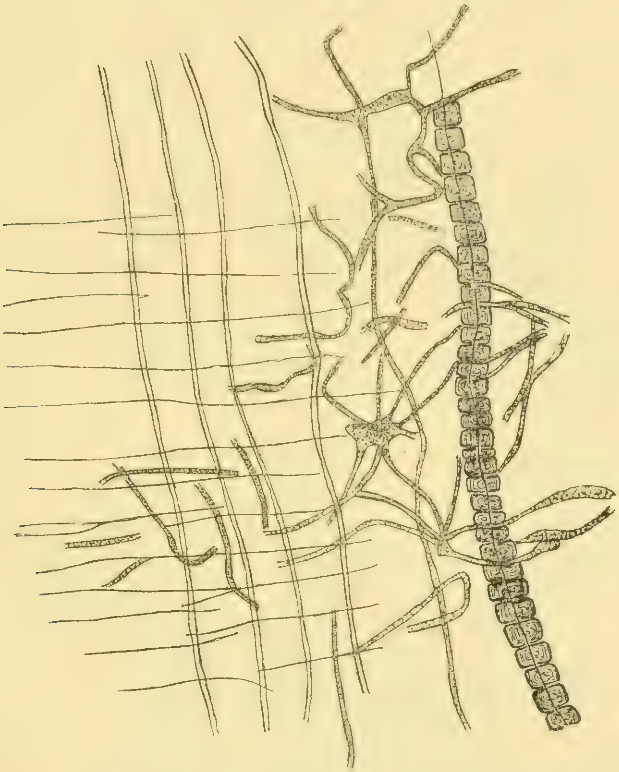


Fig. 3. Longitudinal section showing hyphae passing from medullary rays into vessels. Hyphae pass through without constriction. (Drawing somewhat diagrammatic.) × 160.

The formation of tyloses as a traumatic response is presumably an attempt on the part of the host to check the passage of the fungus through the tissues. In this case it is obviously unsuccessful as the hyphae easily pass through the tyloses (Fig. 4). Failing this function, however, they hasten the death of the branch by preventing the ascent of water.

This investigation of diseased branches explains the symptoms often observed when branches of *Hevea brasiliensis* are affected by Pink Disease. The fungus attacks the wood, vigorously pursuing its course up and down the stem through the vessels. Other vessels are blocked with tyloses for considerable distances while others again are filled with a brown gummy substance. Thus a large proportion of the functional vessels are rendered useless. This results in a serious diminution in the amount of water ascending to the leaves which droop and ultimately die.

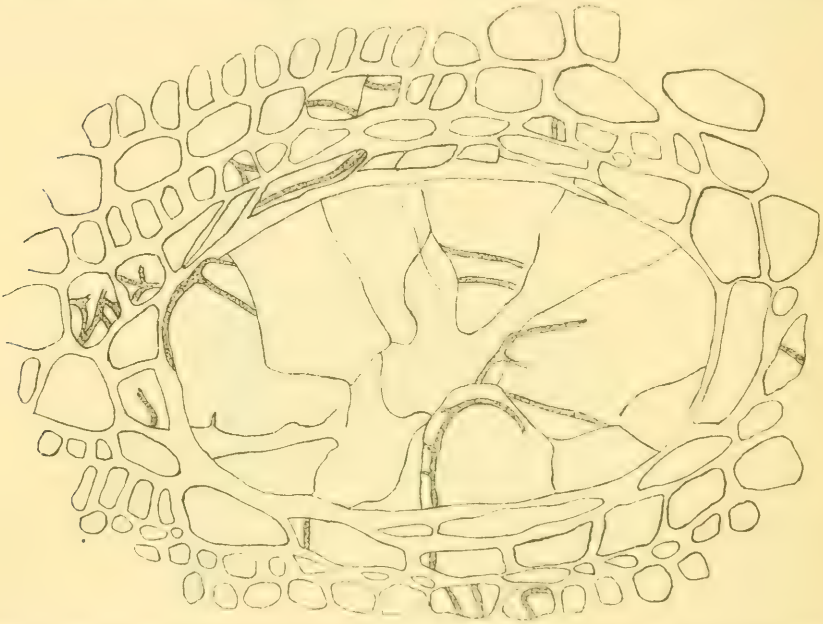


Fig. 4. Transverse section of vessel with thin-walled tyloses, showing hyphae passing through tylose cells. × 160.

Description of the Fructifications of the Fungus.

(*a*) *Basidial stage.* Zimmermann is responsible for placing the fungus in the genus *Corticium* and one gathers from his description and the accounts of subsequent writers that the pink incrustation is the basidial fructification. We found, however, that this stage was sterile in more than 80 per cent. of the large number of cases examined at

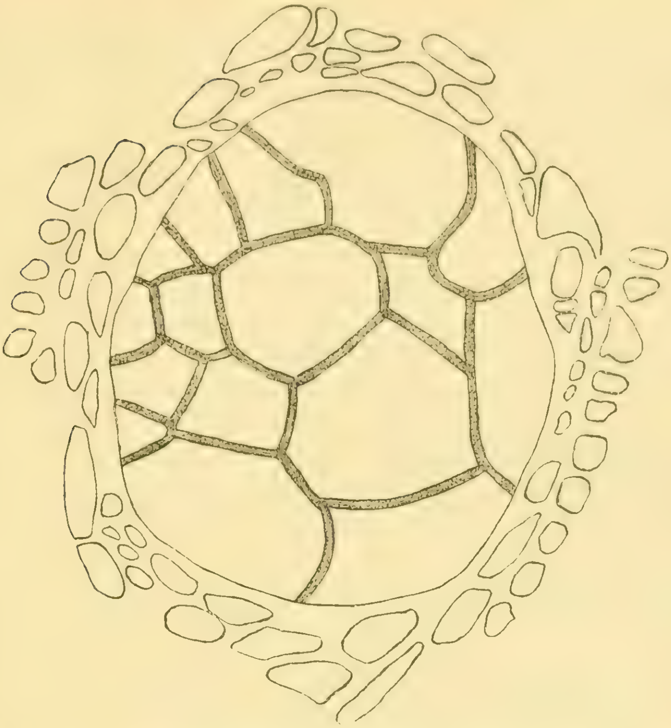


Fig. 5. Transverse section showing vessel with lignified tyloses. × 160.

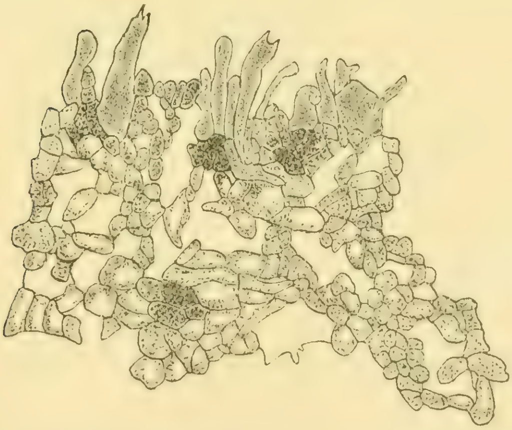


Fig. 6. Section of pink incrustation with basidia. Section 6μ thick and stained with Coton Bleu and Orange G. × 400.

different times of the year. Great difficulty was experienced in finding basidia at all and it was not until after much searching that we recognised the type of incrustation that produced basidiospores. This form is thicker, has a more homogeneous surface, and when dry cracks into larger pieces than the sterile incrustation. It is remarkable that neither Zimmermann nor any other writer has called attention to the fact that the pink incrustation is frequently sterile.

One concludes from Zimmermann's description and figures that a typical hymenium is developed, but according to our experience the basidia are scattered and are irregularly arranged as in Fig. 6. The size of the spores is as given by Zimmermann and the sterigmata are noticeably long. We have not seen the basidia arranged even approximately as they are in Zimmermann's figure. The irregular distribution of basidia reminds one rather of an *Hypochnus* than a *Corticium*. Rant (10) has apparently made no special study of the basidial stage as he copies Zimmermann's figure of the hymenium and agrees with his description of it.

In North America, Stevens and Hall (16) have described a disease of pomaceous fruit trees caused by *Hypochnus ochroleucus*, Noack, which spreads over branches and twigs by means of mycelial strands and kills the leaves by enveloping them. The basidia are scattered and are irregular in form. *Corticium salmonicolor* seems to be more closely related to *Hypochnus ochroleucus* than to other species of *Corticium*. Bernard (3) also has described a disease of tea in Java which he attributes to a fungus named by him *Hypochnus theae*. Though there appear to be certain minor differences between this fungus and *Corticium salmonicolor*, the resemblances in the arrangement of the basidia and the character of the sterigmata and spores are very striking.

(*b*) *Necator stage*. The genus *Necator* was founded by Massee (8) in 1897 for the reception of a single species, *Necator decretus*, which was the cause of a stem disease of coffee in Malaya. It is now known that this is one of the stages of *Corticium salmonicolor*. The *Necator* stage consists of orange-red masses of spores, the individual spores being irregular in shape (Fig. 7) and hyaline when seen under the microscope. Each spore mass is waxy in consistency and it is likely that the spores become separated from one another only in wet weather when they are washed apart.

The mode of formation of these pustules is different from what one would gather by examining Zimmermann's figures, which have also been reproduced in Rant's paper (10). Zimmermann's figures indicate an origin somewhat similar to that of a pycnidium, but we find that the mycelium aggregates beneath the outermost layer of cells of the branch, forming a kind of stroma which by growth ruptures the tissues of the host (Figs. 8, 9). The whole of this stromatic mass becomes converted into spores by the separation of the cells one from the other. The irregularity in the size and shape of the spores (Fig. 7) is due to this peculiar method of spore formation. The dimensions of the spores are 14–20 μ × 8–10 μ. In other species of *Corticium* and *Hypochnus* small sclerotia about the size of pustules of *Necator* are produced and in *Corticium salmonicolor* such sclerotial aggregates may have become modified into spore masses by separation of the constituent cells instead of forming resting bodies.

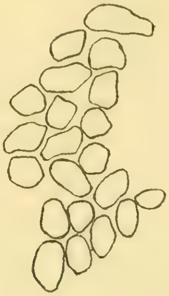
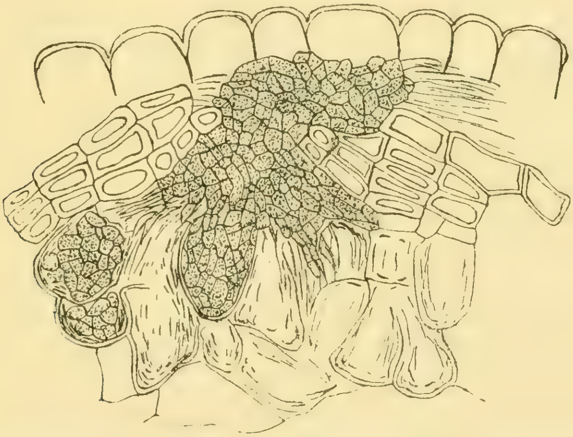


Fig. 7. Group of *Necator* spores, teased out, showing irregular size of spores. ×400.



Figs. 8. Section showing early stage in development of a *Necator* pustule: Stroma forming under the outer layer of cells. ×160.

Necator spores germinate readily in distilled water and in nutritive solutions (Fig. 10).

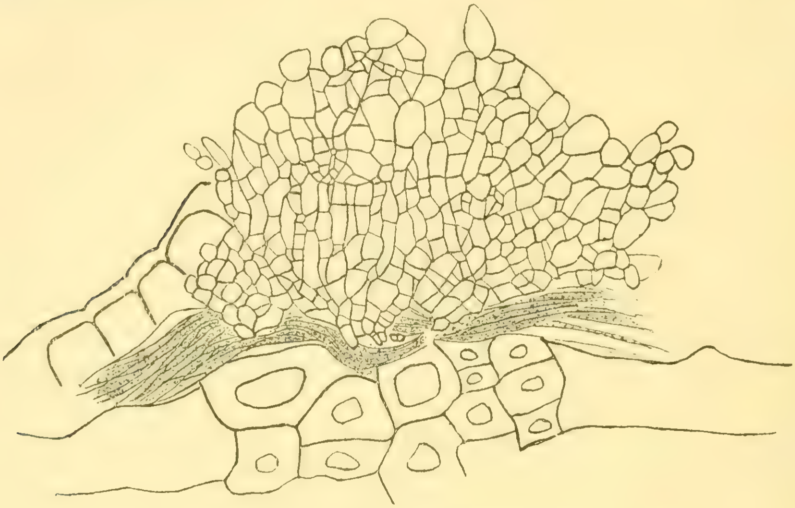


Fig. 9. Section of fully developed *Necator* pustule. ×400.

In Malaya the *Necator* has been found much more frequently than the basidial stage and it is likely that it takes the more active part in the dissemination of the disease.

The other forms of *Corticium salmonicolor* are constantly sterile.

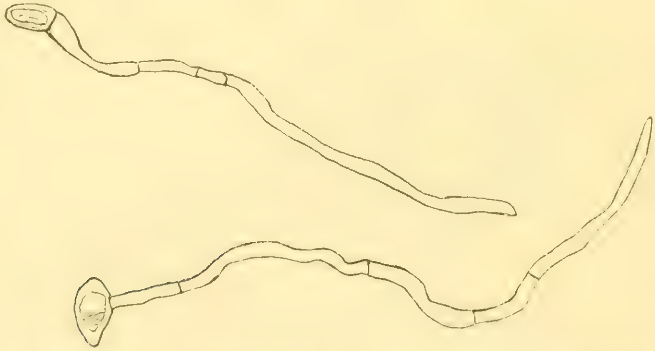


Fig. 10. Germination of *Necator* spores, after 12 hours in damp chamber. ×400.

The fungus is probably chiefly spread by wind, though it is possible that it is also disseminated by red ants and other insects which visit rubber trees. It is possible too that not only spores but also small portions of the sterile incrustation are disseminated by these means.

The incrustation retains its vitality for a considerable time and, as stated above, it cracks into small pieces as it gets older, and these, breaking away, may be carried to other trees in one or other of the ways mentioned.

A species of *Nectria* is often associated with cases of Pink Disease of long standing, but as far as is known at present it is purely saprophytic and develops only after the bark has been killed by *Corticium*.

Pure Cultures of Corticium salmonicolor.

At the beginning of these investigations efforts to obtain pure cultures of the fungus were unsuccessful. We soon recognised that the incrusting form was usually sterile so we gave up attempts to obtain a deposit of basidiospores with which to start pure cultures. Failing basidiospores, small pieces of the pink incrustation were cut out with a sterile knife and placed on slants of salep agar. The fungus quickly developed, but the cultures were usually contaminated. Subcultures started from these were eventually obtained which were probably pure. No further attempt to obtain pure cultures in this way was made because at this stage one of us obtained material in the field which appeared somewhat unusual at the time. This material bearing orange-red pustules was sent to the laboratory and immediately examined, when the pustules were seen to consist of masses of spores, these being the *Necator* form of the fungus which was subsequently obtained frequently.

Pure cultures were obtained from the *Necator* spores. These were teased out on sterile glass slides; some were placed in damp chambers on salep agar, others directly on test tube slants of the same medium. The spores quickly germinated in the damp chambers (Fig. 10); these were kept under observation for three or four days in order to see whether the cultures remained pure. After this, transfers from the damp chambers to test tube slants were made. As these cultures and those obtained by placing spores on test tube slants direct were identical, little doubt remained as to their purity. The mycelium was pure white and did not grow copiously. After a period of about ten days a faint pink colouration appeared in the cultures. The agar cultures were kept for several weeks, but no further development took place. Other cultures were then made by transferring small portions of the mycelium to blocks of sterilised *Hevea* wood placed in tubes (a figure and explanation of the form of tube used is given in Fig. 11). About 50 per cent.

of the attempts to start cultures from small pieces of mycelium were unsuccessful.

The cultures on wood blocks obtained in the first place from *Necator* spores develop quickly. The mycelium grows profusely and remains white for seven to ten days. It spreads over the block and into the cotton wool placed at the base of the tube. Growing in the cotton wool the mycelium begins to turn a pale pink colour which gradually spreads over the whole culture. Subsequently the mycelium in the cotton wool becomes a bright rose colour. The mycelium passes from

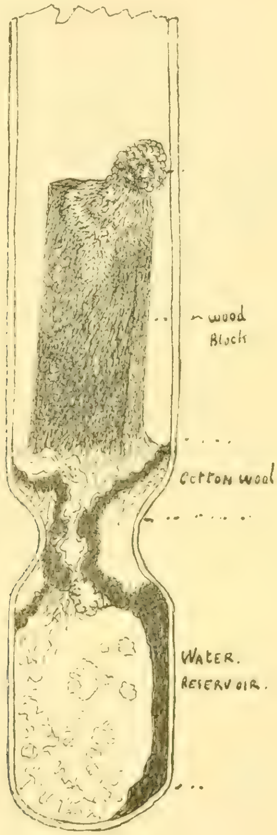


Fig. 11. **Culture tube:** wood block covered with mycelium which has grown into water reservoir covering the water with a film of hyphae on which *Necator*-like masses have formed. Large *Necator*-like masses at top of wood block.

the cotton wool into the reservoir of water where it becomes aggregated.

The cultures started originally from the pink incrustation were now used to inoculate sterilised blocks of *Hevea* wood. The cultures so obtained were identical with those derived from the *Necator* spores so that, apart from other evidence, this alone indicated the connection between the two forms.

The cultures on wood blocks were placed under different conditions to ascertain whether these influenced the development of the colouration. Some were placed in the laboratory in diffuse light, others in direct sunlight, and others in darkness. Rant (10) states that his agar cultures placed in darkness remained white though after illumination for a short time a pink colouration appeared. In our experiments the cultures grown in darkness behaved like those in the light except that the pink colour did not develop so rapidly.

In older cultures the mycelium often turns a dirty brown colour as though some impurity had entered. However, small portions of this mycelium seen by transmitted light show the characteristic colouration. The hyphae in the old brown cultures are closely aggregated, corrugated, septate, much vacuolate, with numerous clamp connections. The hyphae in young cultures are septate, vacuolate, but not corrugated, whilst clamp connections are less numerous.

In one culture placed in bright light and in another kept in diffuse light an aggregation of hyphae took place along the upper edge of the blocks to form a bright pink mass. The aggregation continued until a solid mass half an inch high and half an inch in diameter was formed, attached to the block by a thinner base. It resembled a number of closely attached *Necator* pustules (Fig. 11). Examined microscopically the mass was found to consist of short cells somewhat irregular in size forming a kind of pseudo-stroma.

These *Necator*-like masses are often formed in the cotton wool at the base of the culture tubes and sometimes upon the surface film of mycelium in the reservoir. Their spore-like nature is indicated when small pieces are used to start new cultures upon wood blocks. In every case the mycelium develops copiously within 24 hours, while, as stated above, 50 per cent. of attempts to start new cultures with portions of the usual form of mycelium result in failure. Under natural conditions it is probable that the cells forming these pustular masses would become detached from one another in a manner similar to those forming typical *Necator* pustules.

Rant (10) states that he did not obtain either fruit form in his cultures, but calls attention to the formation of "paraplectenchymatische mycelienknäuel," which he obtained now and again in his cultures and which had been previously observed by Koorders (7) in cultures of *Necator*. The *Necator*-like masses obtained by us probably correspond to these structures observed by Rant and Koorders. Rant confined his attention chiefly to agar cultures, but in our cultures on this medium we did not obtain anything approaching a fruiting form, though our efforts in this direction were not long continued after we found wood blocks so favourable for culture work.

Some of the wood blocks on which cultures had been grown were sectioned. It was seen that the fungus had spread to the centre of the block and that the mycelium had penetrated the elements of the wood in the same manner as in wood naturally infected. Tyloses were absent.

Inoculation experiments.

Rant's inoculation experiments (10) experimentally demonstrated the connection between *Corticium salmonicolor* and *Necator decretus*. Our inoculation experiments to be now described were carried out to test the conditions which favour or hinder the development of the fungus on *Hevea brasiliensis*. Correlated experiments upon other hosts were kept under observation at the same time.

The first series of inoculation experiments was carried out on rubber trees three-and-a-half years of age. The inoculations were made on the 4th of February, 1914, with pieces of the pink incrustation obtained from a diseased rubber tree. The inoculations were covered with cotton wool pads which were moistened every morning for the first three weeks as the weather was dry during this period. The pads were kept in place by rubber bands.

Little rain fell between the date of inoculation and March 10th, but between March 10th and March 16th there were daily showers. The fungus appeared during this wet spell, 13 out of 29 inoculations being successful. Of this number 8 out of 13 (60 per cent.) were obtained upon uninjured parts; 5 out of 16 (30 per cent.) upon wounded surfaces. These results provide further evidence that *Corticium salmonicolor* acts rather as a vigorous parasite on uninjured parts than as a wound parasite.

Between March 16th and April 14th the weather was dry and no rain fell for considerable periods. On the latter date only a few trees showed any trace of the fungus and it appeared that the fungus was dying or was hibernating in the bark.

The inoculations were examined from time to time, but the fungus made no further progress. This result may be attributed to the dry weather which followed the moist spell ending on March 16th. A similar result is often observed on estates, for branches of rubber trees may recover from an attack of Pink Disease if a long spell of dry weather intervenes.

Similar experiments were carried out in the field at the same time on *Coffea liberica*, *Cinchona succirubra*, *Cinchona ledgeriana*, and *Cinnamomum camphora*, six inoculations being made in each host, three being in wounds and three being on unwounded surfaces. Only one successful inoculation was obtained and that on *Coffea liberica* where the cobweb-like form of mycelium appeared over the wound in which the fungus had been inserted.

The results indicate that these hosts are attacked less vigorously than is *Hevea brasiliensis*. These experiments indicate the possibility that small portions of the sterile incrustation may disseminate the disease. These easily break away and are blown about by the wind. Under favourable conditions the mycelium may develop and give rise to a new infection.

Inoculations were subsequently commenced in the laboratory with pure cultures of the fungus. Rubber seedlings and plants of *Gardenia sp.* and *Cinchona succirubra* were inoculated either by tying wood blocks used in growing pure cultures upon the stem, or by placing pieces of the mixture of mycelium and cotton wool from the base of tubes in wounds in the stem or in contact with the stem. Six inoculations of rubber seedlings and of *Cinchona* and three inoculations of *Gardenia* were made on July 10th, but by August 19th no success had been obtained. In these experiments the plants were kept under bell jars. The inoculations on *Gardenia* and *Cinchona* were overgrown with moulds within a week. In several inoculated rubber seedlings, however, the fungus appeared to grow strongly at first, but it soon weakened, and on August 19th the host plants were quite healthy. The experiments indicate that Pink Disease is not likely to attack very young plants of Para rubber.

Inoculations of rubber trees with pure cultures were also carried out in the field, trees standing in an overgrown nursery being used for this purpose. Fairly large branches of the outside trees were inoculated on July 14th with the mixture of mycelium and cotton wool from the base of the culture tubes. Similar experiments were performed at the same time with *Coffea liberica* as host. The following table summarises the results on August 19th:

Name of host	Type of inoculation	No. of inoculations	No. of successful inoculations
Hevea brasiliensis	placed in contact	12	1 cobweb-like form
	placed in wounds	12	1 cobweb-like form
Coffea liberica	placed in contact	4	1 cobweb-like form
	placed in wounds	4	——

Thus successful inoculations of both rubber and coffee were obtained with pure culture material of the fungus.

Rant experimentally demonstrated that *Corticium salmonicolor* does not exhibit the phenomenon of specialised parasitism. Thus this fungus occurring on one host is not limited in infective power to that particular host or a few others, but can attack a wide circle of plants. It passes readily under favourable conditions from one host to another. Only in very wet weather does the fungus spread rapidly, as was obvious in our field experiments. Relatively few of our inoculations were successful and this we attribute to the difficulty of keeping them moist during the spells of dry weather which intervened during the progress of the experiments.

Treatment.

It must be pointed out in the first place that, exceptional cases apart, spraying with fungicides is impracticable and to some extent also useless. Spraying a rubber plantation with trees 30 to 60 feet high is an entirely different proposition from spraying an orchard containing trees only 20 feet or so high. There would have to be something in the nature of a revolution in spraying methods to enable a mature rubber plantation to be sprayed effectively so as to check Pink Disease. Spraying experiments which we recently carried out with two modern machines provided with extension rods show that the maximum height for which they can be used under estate conditions is 25 to 30 feet, and this height is only obtained with difficulty by coolie labour. Longer extension rods have been tried, but the difficulties of manipulation under estate conditions are so great that they cannot be used with success for spraying large numbers of trees. Hence if a mature rubber plantation were sprayed for Pink Disease the upper branches of the trees would remain unprotected and these are the parts most liable to the disease. Again, in view of the regularity of the rainfall in the Federated Malay States a single spraying would be useless. To be effective at all in such a climate, spraying would have to be repeated at frequent intervals.

Spraying with Bordeaux Mixture or Lime Sulphur, preferably the former, might be effective in checking the disease in plantations not more than three years of age if there was danger of it breaking out in epidemic form, but fortunately there is yet no indication of this in Malaya. In Southern India where there is a prolonged dry season, Anstead (2) reports that painting the forks of young trees with Bordeaux Mixture before the coming of the monsoon reduced the percentage of trees affected from 1·34 to ·56, ·07 (three applications of the fungicide were given here), and ·7 per cent. in various cases. In our opinion estates infected to the extent of about one per cent. would be preferably treated by cutting out or by tarring as described below. Another circumstance, which would only occur exceptionally, and in which spraying a limited number of trees might be undertaken, is where the disease is confined to one portion of an estate. In conjunction with treatment within the infected area in the manner described below, it might be advisable to spray carefully a belt of trees around this area as a precautionary measure.

When Pink Disease first appears in a rubber plantation it is usually distributed in a sporadic manner. It is of the utmost importance that the disease should be dealt with vigorously from the outset by cutting off and burning the affected parts. In most plantations where Pink Disease appears for the first time only a few trees are attacked. In such cases diseased branches should be cut off at least two feet below the lowest point where there are obvious signs of the fungus and it is preferable to cut them off flush with the main stem or larger branch.

Where a large number of trees are affected on an estate the manager will probably hesitate before he cuts out the disease in this drastic manner. As an alternative, branches and main stems which appear to have a chance of recovery should be covered with tar for two feet above and below the region over which the fungus is evident. If the disease is dealt with in this way in the early stages many branches and sometimes entire trees may be saved. Even when the fungus has penetrated the bark to a slight extent the external application of tar appears to check its progress. It has been urged that the diseased bark should be removed before tar is applied or even that tar should first be placed over the affected parts, the rotten bark removed, and then tar subsequently applied again. These are excellent ideals and if expense were no object would be strongly recommended. Experience has shown, however, that where tar is applied thoroughly without previous removal of diseased bark good results are obtained as long as the treatment is renewed within a month if necessary. Trees treated with tar for Pink Disease should be examined within a month, and if the fungus has spread, tarring should be tried again. If two applications of tar are found useless in checking the disease the affected parts should be cut out and burnt. On several estates where this mode of treatment has been adopted, Pink Disease has been reduced to a minimum. If tar is used to check Pink Disease it is essential that the work should be done under good supervision, for if done carelessly the money spent on it will be wasted. It is important also that diseased trees should be treated at an early stage. In certain cases, *e.g.* when the leaves of an affected branch have died, it is obviously hopeless to apply tar. The only thing to be done in such cases is to cut out and burn the diseased portions. The use of a concentrated Lime-Sulphur mixture has been tried instead of tar, but it is difficult to check the use of it and it is readily washed off by rain, hence it is not a good substitute and is not recommended.

Planters sometimes have difficulty in burning diseased branches on account of persistent rain. If it is impossible to burn the diseased parts directly, they should be drenched with a 10 per cent. solution of sulphate of copper, removed from the plantation and buried in the ground some distance away from the rubber trees. It must be remembered, however, that there is nothing so good as fire for the destruction of fungoid pests. In this connection mention may be made of the fact that another pink fungus, *Oospora sp.*, which is harmless, usually develops on wood in Malaya a few days after being burnt. This fungus has been several times mistaken for Pink Disease.

Where Pink Disease has appeared in an estate, a pest gang should be formed if not already established and the size of the gang should be such that it can go over the whole estate and treat diseased trees on the above lines once every three or four weeks. Pink Disease develops rapidly and any longer interval is too great to allow of it being dealt with effectively. Where a considerable amount of Pink Disease is present one can hardly expect to eradicate it completely so the expense of maintaining a pest gang must be met. The rubber plantation industry is dependant on the health of the trees so it would be suicidal policy to grudge money for treating disease.

Any plants besides rubber which are affected by Pink Disease in the neighbourhood of estates should be destroyed as the fungus passes readily from one host to another. The manifestations of the disease on other plants are the same as on rubber.

If the measures indicated above are carried out, the disease should be kept under control, but any neglect of it will be dearly purchased. This disease of rubber trees is more common in Malaya at the present time than the die-back caused by *Diplodia* and it is a disease which will have to be watched carefully.

In conclusion we wish to express our thanks to Mr F. de la Mare Norris for kindly making the drawing from which Fig. 11 has been reproduced.

SUMMARY.

1. The distribution, hosts, and mode of action of Pink Disease are described and its importance as a disease of plantation rubber is emphasised.

2. The various forms of *Corticium salmonicolor* are described. It is pointed out that the fungus is not a typical *Corticium* and that the pink incrustation is very frequently sterile.

3. *Corticium salmonicolor* often affects the wood as well as the bark of rubber trees. Its action on the wood is described in detail. The formation of tyloses appears to be a response to the presence of the fungus in the wood.

4. Pure cultures of *Corticium salmonicolor* have been established on salep agar and on *Hevea* wood.

5. Inoculation experiments both with natural material and with the fungus grown in pure culture have been successful.

6. *Treatment.* (*a*) Spraying is not recommended except in particular cases. (*b*) The disease is best dealt with either by cutting out infected branches, or by treating affected parts with tar. Detailed instructions are given as to the manner in which these operations should be carried out by a pest gang.

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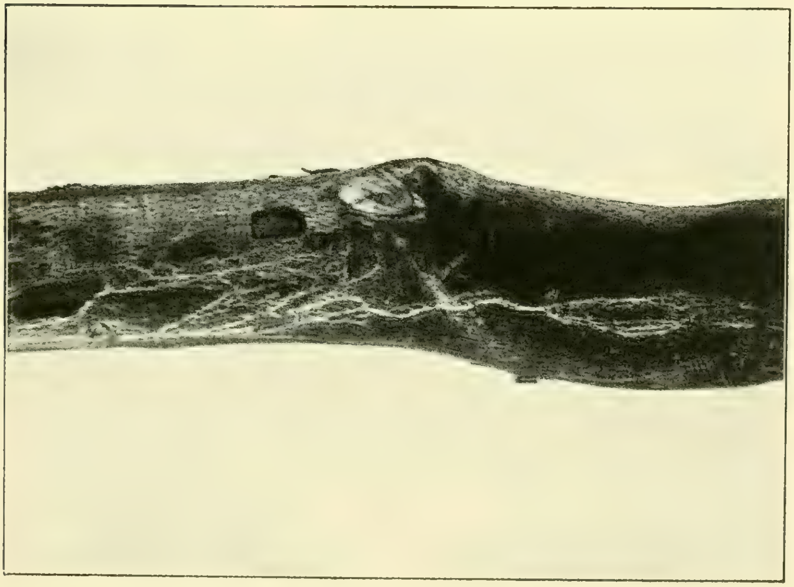

Fig. 1. Photograph of the cobweb-like form of mycelium of *Corticium salmonicolor* on a branch of a rubber tree.

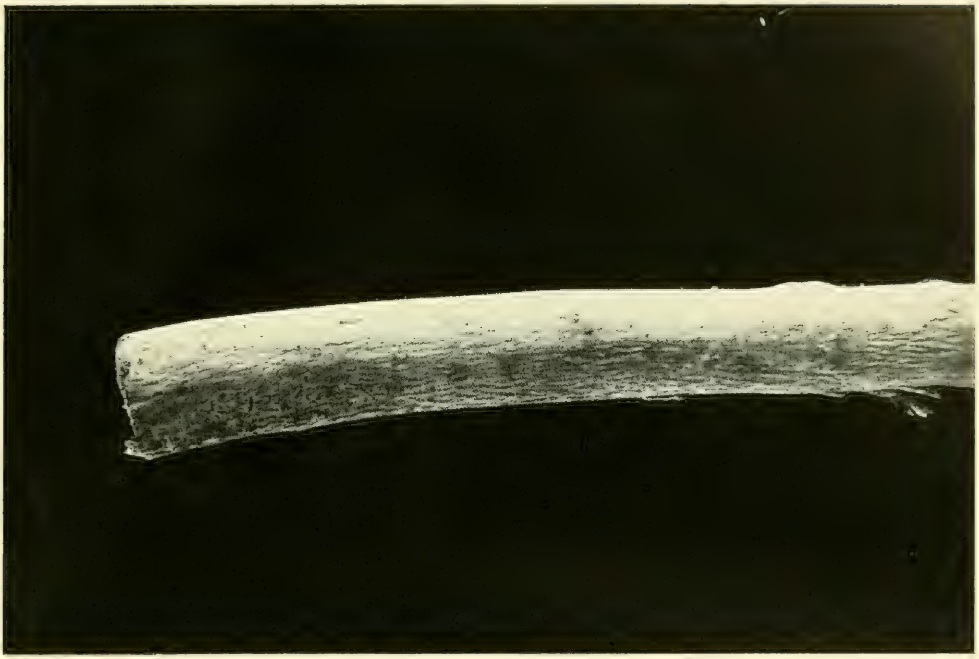

Fig. 2. Photograph of the incrusting form of *Corticium salmonicolor* (to right) and of pustules of the *Necator* stage (to left) on a branch of a rubber tree.

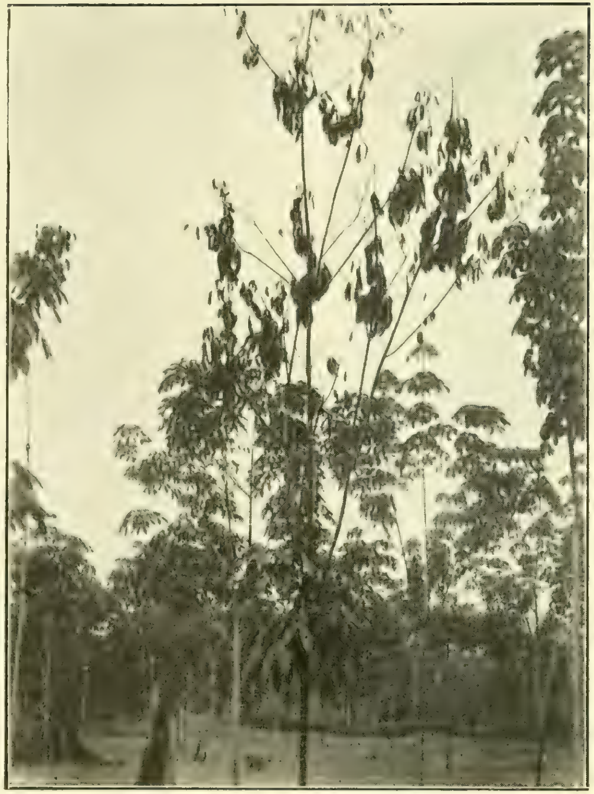

Fig. 3. Photograph of young rubber tree the upper part of which has been killed by *Corticium salmonicolor*.

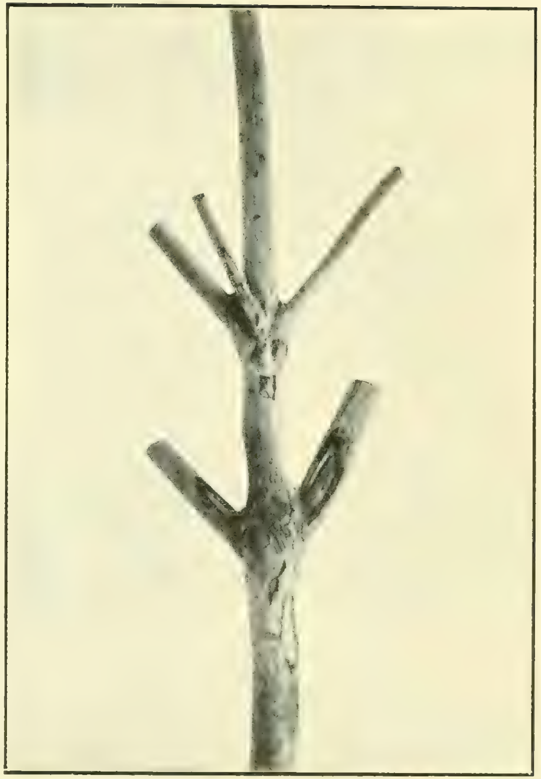

Fig. 4. Photograph of rubber tree bearing "cankers" caused by *Corticium salmonicolor*.

AN ATTEMPT TO MEASURE THE LOCAL AND SEASONAL ABUNDANCE OF THE SWEDE MIDGE IN PARTS OF YORKSHIRE OVER THE YEARS 1912 TO 1914.

By F. W. DRY, M.Sc.,
Fellow of the University of Leeds.

(With Plate XVIII, 2 Text-figures, and 5 Charts.)

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I. INTRODUCTION.

THE object of this paper is twofold, firstly to describe an attempt to measure the relative density in numbers of one particular insect pest at different times in certain selected places; secondly, to examine the extent to which the fluctuations in the figures collected can be accounted for.

It is, of course, notorious that the abundance of insect pests varies enormously in different years and in different localities, but so far as I know little has been done in the way of actually measuring such fluctuations in abundance. Unless such measures can be obtained there is, of course, no definite basis on which to correlate differences of abundance with differences of conditions. Various factors may be suggested as of importance in determining the degree of prosperity of an insect, but without a statistical basis it must necessarily be difficult or impossible to discuss such factors with precision.

The only way in which greater precision can be ensured appears to be to undertake a census over a period of years and preferably over an area which includes districts which differ amongst themselves in the conditions to which the insect is exposed in them. The conclusions which are drawn from such a census should then so far as possible be subjected to the test of experiment.

The present paper is an account of work carried out on those lines on the Swede Midge, *Contarinia* (*Diplosis*) *nasturtii*, Kieff. The country worked in was the area in the East Riding of Yorkshire shown on the accompanying charts, and also a small area round Garforth in the West Riding.

My object was to obtain a figure for each of the districts into which I divided my area, which should be roughly proportional to the number of Swede Midge individuals per acre of swedes. With many crops and many insect pests methods like those shortly to be described could not be used. In the case of the Swede Midge, however, a combination of fortunate circumstances makes it quite practicable to carry out a fairly accurate census, and to do so, if desired, two or three times in one summer. These particular circumstances are briefly as follows:

1. The number of swede plants in a row can easily be counted.

2. It is easy to recognise at a glance the characteristic distorted appearance of the swede plant produced by Swede Midge attack.

3. Signs of Swede Midge attack on a plant last, as a rule, about six weeks after the eggs have been laid.

4. Swede Midge maggots remain on the plant upon which they have been born until they have completed the feeding stage. They do not move from one plant to another.

5. So far as I am aware the Midge never kills a plant. However badly a plant may be attacked, it remains alive and shows, by its distorted appearance, that it has been attacked.

6. The several broods of the Midge do not succeed each other too quickly or overlap too much for it to be impossible, by choosing the right time, to make a census for each brood in an area such as that in which I worked.

II. *Life-history of the Swede Midge.*

The life-history of the Swede Midge and the distortion which it causes to the swede plant are described by Mr T. H. Taylor in *Cabbage-Top in Swedes*[1]. The following short account is based on that in Mr Taylor's paper.

The flies are first found in swede fields in June. The eggs are laid in strings and clusters mostly near the base of the leaf-stalks, but some on the leaf-blades; the younger leaves are almost always the ones to be attacked. The eggs hatch in about four days and the maggots feed on the superficial tissues. The larval stage lasts about three weeks. When full grown the maggots go down into the ground and the flies come out, in the case of the summer broods in from two to three weeks. The normal number of broods for the season appears to be three, although in the hot summer of 1911 there were four broods. The winter is spent in the soil.

Mr Taylor describes and figures the distortion to the plant caused by the maggots. (Cf. figs. 2 and 3, Plate XVIII, of the present paper.) He says "In three or four days after the larvae have started to feed the plant begins to show signs of being damaged. The stalks of the affected leaves become swollen, and bending sharply inwards across the top of the plant, press upon and compact the terminal bud. Moreover, the leaf-blades, at those areas where the larvae are feeding, become delicately crumpled, thus resembling the leaves of a savoy cabbage." Mr Taylor also proved that the Swede Midge was one cause of what he calls "many-neck" in swedes, that condition in which the plant has several necks or main shoots instead of the single one of a normal plant.

My own observations agree with Mr Taylor's account. I think, however, that in 1913 the time elapsing between the maggot's entering the soil and the emergence of the fly was often as long as a month. It will be seen that in this paper I am concerned only with the crumpled-leaf condition, and not with "many-neck." Besides the Swede, *Brassica*

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[1] Publications of the Yorkshire Council for Agricultural Education, No. 82.

rutabaga, Mr Taylor has found the following plants to be attacked by the Swede Midge:

Brassica napus	Turnip.
B. rapa	Rape.
B. oleracea	Cabbage.
B. sinapis	Charlock or Wild Mustard.
Raphanus sativus	Radish.
R. raphanistrum	Wild Radish.

Turnips I have found by observation to be attacked only to a very small extent. Rape occupies an area small compared with that of swede crops. Cabbage and Radish occupy a very small acreage compared with that of swedes. Wild Radish is not a very abundant weed, but Charlock is found in plenty.

Bezzi and Kertesz in their *Catalog. Palaearc. Dip.* give as host plants of the Midge,

Nasturtium palustre	Marsh Watercress.
N. silvestre	Creeping Watercress.

These plants are found in wet situations in Holderness, but whether they are attacked there by the Midge I cannot say.

The relation of the Midge to wild hosts is a question which I have not yet investigated. But on p. 94 I explain why I believe wild hosts are not of much importance in determining the abundance of the Midge in a district.

III. *Methods.*

Swedes are grown in rows which are from 24 to 28 inches apart, and in singling the plants are left about 12 inches apart. It is therefore perfectly easy to count the number of swedes in a row. It is also quite easy to tell at a glance which plants have been attacked. Moreover, the maggots spend the whole of the feeding period within a very short distance of the point where the eggs were laid. They do not move from one plant to another. And the distortion brought about by the Swede Midge can easily be distinguished from that caused by other insects. As Mr Taylor says (p. 12), the leaves "retain to the end discoloured traces of the injury, and since the crumpled character persists, it follows that the observer can readily recognize, by their leaves, swede plants that have been attacked by the Midge, although neither flies, eggs, nor larvae may be present." I did, of course, come across plants which I was not able to say with certainty had or had not been attacked, but as I made all the observations myself there was not more than one personal equation as a source of error. And I took it as a guiding rule in estimating percentages of attacked plants, when the maggots had left the plants, in doubtful cases to count as attacked only those plants of which one or more leaves showed a scar as well as the supposed crumpled appearance. This would mean that a few slightly attacked plants were not counted as attacked, but it was as good a rule as I could find. I am confident therefore that the error due to failure to recognize attacked plants was a very small one. Thus by simply counting the number of attacked plants in a row the percentage in that row could be calculated.

I have found by a laboratory experiment that one female fly can lay enough eggs to bring about the characteristic crumpled leaf appearance. And after examining a number of attacked plants with eggs or maggots on them, I think that the average number of eggs laid on a plant may be put at about fifteen, that estimate being, I believe, low rather than high. I have estimated the number of eggs in a few midges cut into microtome sections to be about a hundred. This suggests that one midge may infect from one to, say, ten plants. Several times I have found either two or three attacked plants adjacent to each other in a row of swedes, separated from the next attacked plant in that row be several hundred plants. It is probable, therefore, that these two or three plants were selected by one and the same female for oviposition. The number of swede plants in an acre after singling is about 20,000. From these data, therefore, if it served any useful purpose, we could calculate the number of female flies which would be needed to infect any particular percentage of plants in that area.

I should point out that in the operation of singling, which is performed, as a rule, from four to six weeks after sowing, a large proportion of the plants are knocked up with the hoe, and die. In early sown fields singling may be finished before any or many of the flies have made their appearance. In later sown fields, on the other hand, some plants will be attacked before and some after singling, when the number of plants is greatly reduced. And sometimes in the latest sown fields singling may not even commence until all the flies of the first brood are dead. This must be one reason why, as will shortly be shown to be the case, the figures from late sown fields are lower than those from early sown fields, for all the figures were obtained after singling had taken place.

My usual method was to find the number of attacked plants in each of two rows widely separated from each other in each field visited. If the figures from the two rows agreed fairly closely, I took the percentage of attacked plants calculated from those two rows as the percentage for the field. If there was a considerable difference between the two figures, I would examine one or more further rows. Sometimes in the Brood II or Brood III surveys, when it was necessary to hurry through the survey as quickly as possible, I would examine only a single row. This I took care was about half way between the two rows examined in the first survey. I also found out whether each field was sown in May or June by asking people I met in the fields, or failing them I would estimate by eye, a thing which I was able to do with very fair accuracy. In this way a figure for the percentage of attacked plants and the approximate sowing date for each field visited were obtained. Each year I examined about 200 fields, examining the same fields in all the surveys of any one year.

I should point out that as the leaves grow from the heart of the plant to the outside and finally fall off, signs of attack do not last for more than about five or six weeks.

In 1913 in the East Riding the three broods of that summer did not, so far as I was able to tell, overlap. In 1914 they did overlap. I call the second survey the Brood II survey, and the third the Brood III survey, because I am sure that flies of those particular broods were respectively responsible for the greater part of the attack in each case. But it is not impossible that in the second survey some plants were counted which had actually been infected by flies of the third brood, and that in the third survey some plants were counted which had been infected by flies of a fourth generation. The two later surveys which were hurried through quickly are therefore surveys of the numbers of plants which could be seen to have been attacked at the stated times at which the surveys were carried out. The dates of the surveys and the tests applied to their accuracy are given in the Appendix. These tests show, I consider, that the figures obtained in my five surveys were sufficiently accurate for the purposes to which I put them. After the conclusion of the last survey charts were constructed, one for each year, on which all the fields examined were marked. The East Riding area was then divided into eleven districts and Garforth made a twelfth. The ground occupied by the town of Bridlington is not included in any district. All small plots near to swede fields of the previous year were omitted from calculations for a reason which will appear shortly.

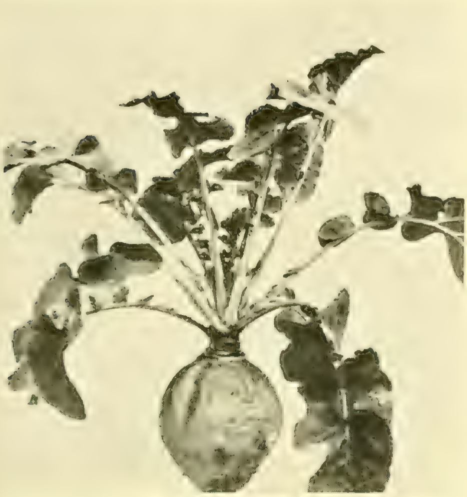

Fig. 1. Sound swede plant.
(From Mr Taylor's *Cabbage-Top in Swedes.*)

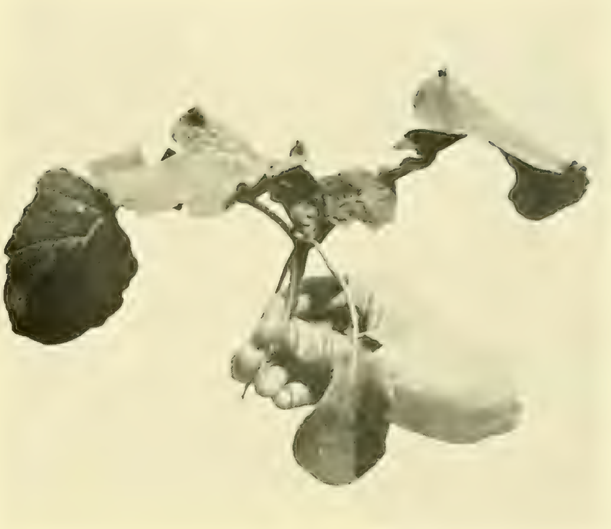

Fig. 2. Young swede plant, showing early stage of crumpled-leaf condition.

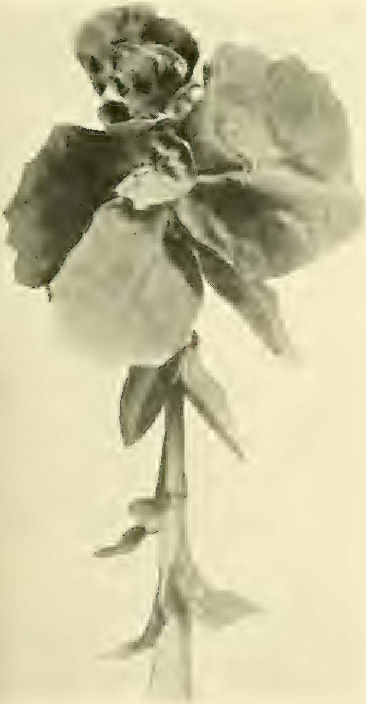

Fig. 3. Swede leaf, showing later stage of crumpled-leaf condition.

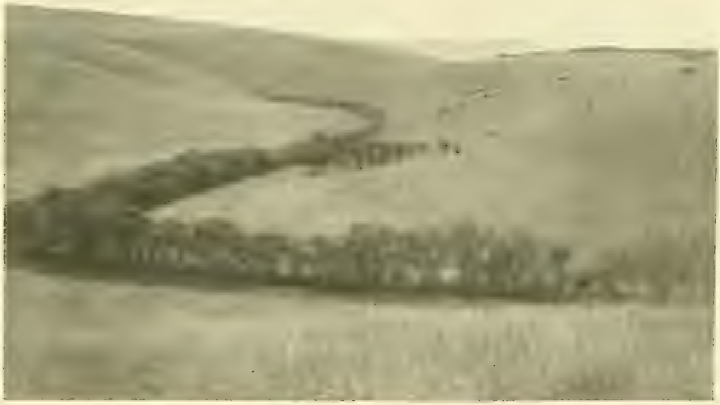

Fig. 4. Cowlam Well Dale. A typical narrow wold valley (District V). August, 1913.

For each survey in the different districts a figure was calculated which may be taken as a measure of the relative numbers of the Midge per acre of swedes, and may be called the **Swede Midge index**. This figure was obtained in the following way. For each survey the average percentage of attacked plants in the fields examined in each district was calculated. In speaking of the percentage of attacked plants in a field the abbreviation "% CL" is often used, meaning the percentage of plants showing the crumpled-leaf condition. An average figure is therefore sometimes referred to as the "average % CL." When a calculated average % CL for a district was between two whole numbers, the higher whole number of the two was taken as the index. For example, whether the figure was 7·1, 7·6, or 8·0, the index was taken as 8. The indices are tabulated in Tables 2 and 3 and plotted on Charts 1 to 5.

In my East Riding area there are two chief types of country. In the two Holderness districts the whole area lies below the 100 ft. contour and is quite flat. The boulder clay soil is deep and strong and requires artificial drainage. The Wold part of the area is higher, though nowhere as high as 600 ft., and contains numberless valleys (see fig. 4, Plate XVIII). A thin, comparatively light soil covers the porous chalk, and artificial drainage is unnecessary.

From figures published by the Board of Agriculture[1] I calculate that the proportion of land under turnips and swedes in the East Riding is about one acre in ten. This proportion is higher than in any other county in Great Britain. Swedes are probably grown on about one acre in twenty.

IV. *Statement of results.*

The more striking features of the appended tables may be briefly summarized.

1. In May sown fields the attack of the Midge is greater than in June sown fields.

2. The first brood of 1912 was a very large one. That of 1913 was a very small one. The progress of the Midge in the East Riding area from the first brood of 1913 to the third brood of 1914 is shown in a simple manner in the following table:

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[1] Abstract of Agricultural Returns for Great Britain, collected 4th June, 1910.

		1913 Brood I	1913 Brood II	1914 Brood I	1914 Brood II	1914 Brood III
Number of districts with indices	1, 2, or 3	11	8	7	4	3
,,	4, 5, or 6	—	3	4	7	2
,,	7, 8, or 9	—	—	—	—	3
,,	10 or more	—	—	—	—	3
Total number of districts		11	11	11	11	11

At Garforth the progress was more rapid. The indices were:

1913 Brood I	2
1913 Brood II	No survey
1914 Brood I	22
1914 Brood II	32
1914 Brood III	76

3. In the East Riding area the indices are on the whole higher in the northern part of the area than in the southern. If we divide the whole area into two parts, a northern part containing districts I, II, III, IV, V, and VI, and a southern part containing districts VII, VIII, IX, X, and XI, and calculate for each survey the average percentages of attacked plants in the fields of these two parts, we get the following figures:

	Northern part		Southern part	
Survey	Number of fields examined	Average % CL	Number of fields examined	Average % CL
1913 Brood I	75	1·3	76	0·8
1913 Brood II	75	2·7	76	2·8
1914 Brood I	80	3·1	109	1·6
1914 Brood II	80	4·3	109	2·4
1914 Brood III	80	9·7	109	4·0

4. The coast districts in the East Riding area have on the whole distinctly lower indices than the inland districts. On dividing the area into a coast region, including districts I, II, III, and X, and an inland region, including districts IV, V, VI, VII, VIII, IX, and XI, and finding the average percentage of attacked plants in the fields of these two regions, we get the following figures:

	Coast region		Inland region	
Survey	Number of fields examined	Average % CL	Number of fields examined	Average % CL
1913 Brood I	53	1·1	98	1·2
1913 Brood II	53	1·4	98	3·5
1914 Brood I	52	1·8	137	2·3
1914 Brood II	52	1·9	137	3·7
1914 Brood III	52	3·2	137	7·6

These differences in abundance of the Midge of the different broods and in different localities stand out clearly enough. In the following section I try to explain them.

V. *Explanation of results.*

(*a*) *Conditions determining the percentage of plants attacked by flies of Brood I of the Swede Midge in a given field.*

Apart from conditions affecting the whole of any particular Swede Midge District in any particular year the following conditions are important in determining the percentage of plants attacked by Brood I of the Midge in any given field.

(1) Date of sowing.

Early sown fields are attacked worse than late sown fields. This is shown in Tables 2 and 3. As already explained singling is doubtless in part responsible for the figures collected in June sown fields being lower than those from May sown fields. But this explanation will not account for the complete absence of attacked plants from many June sown fields, which I frequently found to be the case, for swede hoers are almost all unaware of the existence of the Midge. The Midge does not infect the plants until they get into rough leaf. The flies of the first brood appear in June, a few sometimes in July. Consequently early sown fields are exposed longer to attack than late sown fields.

(2) Distance from a swede field of the previous year, and

(3) Size of field.

In 1913 and 1914 I found that numerous small plots of early sown swedes next to swede fields of the previous year had percentages of infected plants high compared with the percentages in other fields in the same district. The following are examples of such 1913 fields:

	% CL	Highest other % CL in district
A. District IX	6	3
B. ,,	11	
C. ,,	30	
D. District VII	19	8
E. ,,	24	

The graph, Fig. 5, is of interest in this connection. In this graph the % CL is plotted for different rows, of which the numbers are given, in a 1913 swede field next to a 1912 swede field. The left side of the graph represents the side of the 1913 field near to the 1912 field, the right side the side of the field remote from that field. Similar graphs could be drawn for other similarly situated 1913 fields and for 1914 fields near to 1913 fields. The graph shows a quick drop in % CL as the distance from the 1912 field increases, the first few rows being badly attacked, while the middle and far side of the field are not much, though a little, worse attacked than any part of a field not near to a 1912 field. Thus in a small field near to a swede field of the previous year, if both fields were early sown, the percentage of attacked plants is usually high. But in larger fields so situated the attack is to a large extent concentrated on one side and the field as a whole is not so badly attacked.

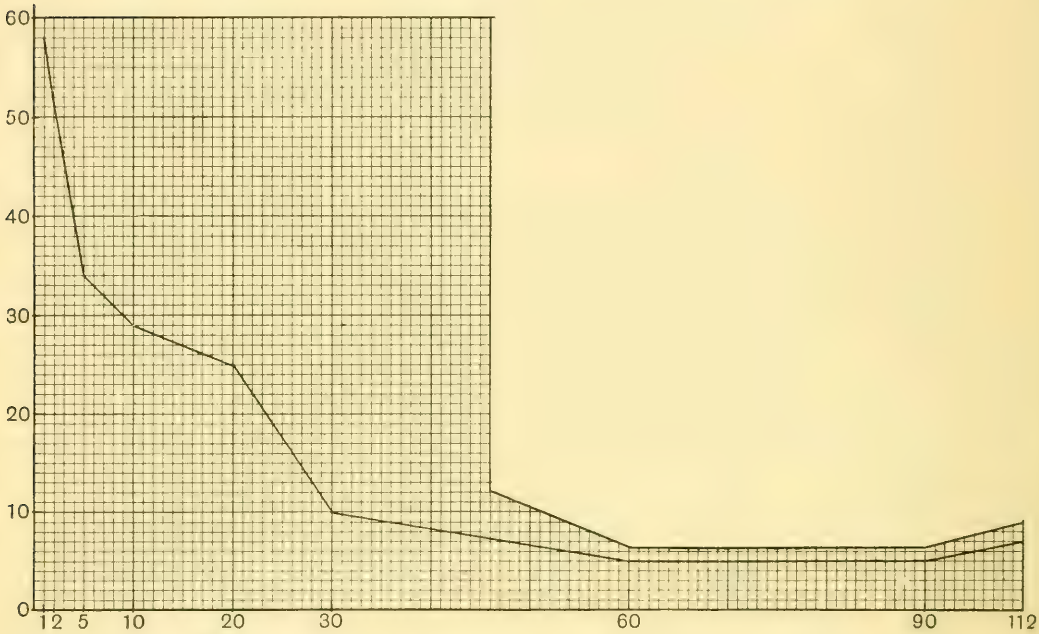

Fig. 5. Graph showing distribution of plants infected by the Swede Midge (Brood I) in a 1913 swede field adjacent to a 1912 swede field.
The rows are numbered from the side of the field next to the 1912 field.

(*b*) *Evidence that flies of the later broods do not always remain in the field in which they have spent the larval and pupal periods.*

Before discussing various points which follow it is important to know whether any appreciable number of flies of the later broods leave the field in which they have spent the earlier stages of their life-history.

It is obvious that the flies of the first brood have to move a greater or less distance to find swedes as it is extremely unusual for swedes to be grown on the same ground in two consecutive years. And I obtained evidence in 1913 that many flies of the second brood did lay their eggs in fields in which they had not spent the pupal stage. Many fields, chiefly late sown ones which missed the first brood altogether, were attacked by the second brood, often having a percentage of infected plants quite high for the year 1913. The following are a few examples taken from a considerable number of such cases:

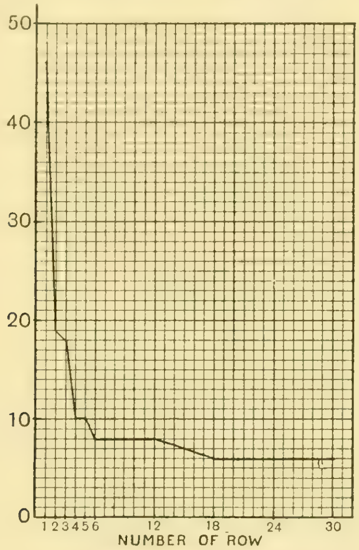

Fig. 6. Graph showing distribution of plants infected by Brood II of the Swede Midge in a late sown 1913 swede field which was not attacked by Brood I, situated near to an earlier sown 1913 swede field which was attacked by Brood I.

The rows are numbered from the side of the field near to the earlier sown field.

		Date of sowing	Brood I % CL	Brood II % CL
F.	District I	May, last week	0	3
G.	„ IX	June, first week	0	9
H.	„ IV	June, second week	0	3
I.	„ VII	June, second week	0	10
J.	„ VII	June, third week	0	20

Clear evidence of the actual movement of flies from one particular field, comparatively early sown, to a later sown field, which missed the first brood altogether, is given in the graph, Fig. 6. Here the % CL

(Brood II) is plotted for various rows in Field K at Driffield, sown June, last week, which was next to Field L, sown June, first week, which was attacked by the first brood of the Midge. The two lots of swedes were separated by a hedge and about thirty yards of barley. The graph shows a very marked drop in the percentage of plants attacked in Field K as the distance from Field L increased.

In 1914 the evidence of such movement was not so clear.

(*c*) *Factors determining the Swede Midge index in different districts.*

The indices for each district in each survey are given in Tables 2 and 3, and plotted on Charts 1 to 5. On the charts for the Brood II and Brood III surveys I have also shown in which districts the index exceeded the index of the previous survey and in which districts the index was not greater. In Chart 5 the indices are the highest and the differences between the various districts are greatest.

The two factors which seem to be most important are:

(1) Average date of sowing,
(2) Distance from the sea.

(1) Average date of sowing.

In the northern part of my East Riding area, as I have shown (p. 88), the Midge is more numerous than in the southern part, and a glance at the charts will emphasize this fact. Mr Henry Ullyott, C.C., farmer and agricultural valuer, has kindly provided me with what in his experience are the average sowing dates for these two parts of my area. For each of the districts in the northern part the estimate which he gives is the last week in May; for all those in the southern part, the first week in June. In my survey work I examined fields just as I came to them, and the numbers of May sown and June sown fields which I visited in the northern and southern parts are as follows:

	1913		1914	
	Number of May sown fields	Number of June sown fields	Number of May sown fields	Number of June sown fields
Northern part	52	23	69	11
Southern part	27	49	55	54

In 1914, Mr Ullyott tells me, sowing was earlier than usual throughout the East Riding area. At Garforth, where the Midge is more numerous than in the East Riding, sowing is earlier. The average date there is the third week of May. Neither in 1913 nor 1914 did I come across any fields sown later than the last week in May.

I have already shown that May sown fields are worse attacked than

June sown fields, so that the larger number of June sown fields in the late sowing districts helps to keep the indices low in those districts, but a glance at Tables 2 and 3 will show that the average numbers of attacked plants for May sown fields in the early sowing districts are higher than for May sown fields in late sowing districts.

There is no evidence of any brood of the Midge in Yorkshire earlier than the first brood which attacks swedes. The question at once presents itself, therefore, what is it which brings to a close the long winter's rest? It cannot be mere lapse of time, for if it were we should never find an interval between two broods of one summer during which flies are relatively scarce or not to be found at all. During the Christmas and Easter terms of 1913—14 and the Christmas term of 1914 I have kept maggots in sand or soil indoors at average temperatures of from 55° F. to 70° F., and have reared a number of flies from them; but from maggots kept out of doors under the same moisture conditions, I have not reared a single fly. This suggests that temperature is an important factor, if not the only one, in bringing about the emergence of the first brood of midges. Now from observations in the field in 1911 on the length of time during which the flies of a brood could be found, it seems unlikely that the average lifetime of the fly is more than a week. We are thus able to understand the importance to the Midge of the average sowing date of swedes.

Swedes grow faster in the later sowing parts of my East Riding area than in the earlier sowing parts, doubtless owing to slightly higher temperature. Moreover, throughout my East Riding area, earlier sowing than is actually the practice is prevented by the fear of Leaf Mildew which attacks early sown swedes more than late sown ones. This mildew may therefore be looked upon as, indirectly, a limiting factor of the Swede Midge.

It is interesting to find that in the United States the chief way of combating that dangerous Cecidomyiid enemy of wheat, the Hessian Fly, is deliberate late sowing.

There may, of course, be other factors contributing to the greater success of the Midge in the early sowing districts. One factor may be the proportion of land under swedes. This is somewhat higher on the Wolds than in Holderness, because a four course rotation is the rule on the Wolds, while a five course one is the practice in Holderness. In Holderness, too, more mangels are grown as a root crop and the proportion of grass land is larger. Soil differences may be another factor. But it is especially notable that the Little Weighton wold district

(District XI), where the average sowing date is the first week of June, has very low indices.

I am also aware that the presence or absence of the cresses *Nasturtium palustre* and *N. silvestre* may prove to be a factor of importance. J. F. Robinson[1] records these cresses from the Holderness part of my area, but not from the Wolds. This point requires investigation.

(2) Distance from the sea.

The Swede Midge is less numerous in seaside districts than in inland districts. I have shown this to be the case on p. 88 and the charts show it quite clearly. The frequent failure of the Midge to increase in number from one brood to another in these seaside districts may be noted.

An explanation of these low indices was suggested to me by an accidental observation at Garforth early in August 1912. A bowl of water happened to be standing about a foot and a half from the ground near to a swede field. Within five minutes of each other two flies got on to the surface of the water in this bowl. As soon as a Swede Midge fly drops on to a surface of water it is helpless. This suggested to me that in districts near the sea the Swede Midge indices are kept low by the drowning of flies in the sea. Flies of the first brood must move greater or less distances if they are to find swedes. I have shown that some flies of the later broods leave the fields in which they have spent the earlier stages of their life-history, so that the later broods could suffer as well as the first. The midges are by no means strong fliers for if one is liberated in quite a gentle breeze it appears to be blown helpless by the wind. Two fields in the Flamborough district (District III) in 1913 do, I think, throw light on the problem. They were comparatively small plots next to ground which had grown swedes in 1912. The sowing dates and percentages of plants attacked by Brood I were:

M. May, 3rd week. 10 %.
N. May, 3rd week. 6 %.

In the other fields in the district the highest percentage of attacked plants was 2·5 %, and the average 0·7 %. These figures suggest that when the flies have only a short journey to make near the sea many of them accomplish it safely, but that when they have to move further in search of swedes many of them are lost in the sea.

I have examined wind records kept at Bridlington for the period June 1st to Sept. 30th of 1913 and 1914. In 1913 the frequency and strength of winds which would tend to blow midges into the sea and also from inland to seaside districts were about equal with the frequency and strength of winds which would tend to blow flies from seaside to inland districts. In 1914 the seaward winds were about twice as frequent and of somewhat greater force than those blowing off the sea.

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[1] *The Flora of the East Riding of Yorkshire*, 1902.

It has been objected that even if many flies were drowned in the sea their places would be taken by flies which moved from inland districts; such movement probably does take place. A glance at the five charts will show, however, that since the time of the first brood of 1913, when the Midge was reduced almost to a uniform low level in the East Riding and at Garforth, the tendency has been for differences between the indices of the richer and the poorer districts to become greater. This is in spite of whatever migration there may have been from one district to another.

(*d*) *Seasonal variations in the abundance of the Swede Midge.*

The fluctuations in abundance of the Midge from the first brood of 1912 to the third brood of 1914 are shown in Table 1, and for 1913 and 1914 have been summarized on p. 88.

The summer of 1911 was exceptionally hot; that of 1912 exceptionally wet and cold; that of 1913 was unusually dry with the temperature about normal; and in the summer of 1914 the rainfall was about normal and the temperature a little above the average.

In the summer of 1911 the Midge increased enormously. The first brood of 1912 was a very large one, but at the end of the summer maggots were very scarce, and the first brood of 1913 was a very small one. The Midge made slow progress in 1913, but rather greater progress in 1914.

Doubtless there is an important relation between the weather and the prosperity of the Midge. In Table 1 it will be seen that the progress made by the Midge in a summer varies roughly with the average mean air temperature or with the number of hours of bright sunshine. With the rainfall figures there is no such clear parallel. Rain probably is harmful to the Midge by washing eggs off the plants and by interfering with flight and egg laying. In hot weather and in the warmer parts of the day the flies are more active than when the temperature is lower. In a hot summer such as 1911 the time taken by the life cycle is shorter than in a cooler summer such as 1913. In some preliminary experiments the time elapsing between the going down into the soil of maggots and the emergence of the flies was found to be shorter at comparatively high than at comparatively low temperature. But I do not think it profitable to discuss further the effect of weather upon the Midge until I have investigated the relation between the Midge and a Proctotrypid parasite which I reared from some maggots in the summer of 1914, and have searched for further parasites. For it is quite possible that the slaughter of 1912 was largely performed by parasites, and would have taken place even if that summer had been as fine as the preceding one.

It is recorded for the Hessian Fly that a summer in which the fly multiplies greatly is often followed by one in which it is extremely reduced, and this reduction is attributed to parasites. P. Marchal records[1] that in Vendée in 1894 the Hessian Fly increased greatly during the summer, but that it was very reduced in numbers in 1895. His explanation is that this was largely due to parasites which had also increased in 1894, though they commenced to hibernate earlier than the Hessian Fly. The flies of the last brood of 1894 were thus able to lay their eggs without the larvae hatching from those eggs being attacked by the parasite. But in 1895 the parasites hatched out in such numbers that they were able to kill a very large proportion of the maggots of the first brood of the Hessian Fly. A similar thing may have happened to the Swede Midge in 1912. I am therefore aware that the relation between the Midge and the weather is very likely not so simple or direct as it may appear to be from a glance at Table 1.

(*e*) *Other conclusions drawn from a comparison of the figures collected in the five surveys.*

In 1912 although I did not carry out a systematic Brood-I survey I was able to see that there were large differences between the percentages of infected plants which I found in different districts. But in 1913 the first brood was reduced almost to the same dead level over all the country in which I worked, at Garforth and in the East Riding. This general levelling may have been due to two causes:

(1) The killing of a larger proportion of the Midge individuals in the more densely populated districts.

This quite possibly happened, though I have no evidence on the point.

(2) The migration of flies from more densely to less densely populated districts.

Such movements of flies probably did take place, for I have shown that while flies of the first brood must move greater or less distances if they are to find swedes, flies of the later broods also sometimes move away from the fields in which they have spent the pupal stage. So one would naturally expect more flies to move from rich to poor districts than from poor to rich districts, though I have already pointed out that any such migration which may take place does not occur to an extent large enough to prevent there being marked differences between the indices of different districts. A comparison of Charts 1 and 5 will emphasize this point.

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[1] "Les Cécidomyies des Céréales et leurs parasites," *Ann. Soc. Entom. France*, 1897, Vol. LXVI, p. 12.

If, however, it is the case that more flies move from the Holderness Inland district to the Holderness Coast district, one conclusion seems to follow. In not a single survey is the average percentage of attacked plants in the Holderness Coast district as high as 1 %. From 1913 Brood I to 1914 Brood III the Garforth index has increased from 2 to 76, and the Midge has made considerable though less marked progress in other districts, but in the Holderness Coast district it has made absolutely no progress. I therefore suggest that were it not for the continual immigration of flies the Midge would soon cease to exist in this district. This may be the case too in the Flamborough district. And probably the Filey and Bempton figures are kept higher by immigration than they would be if those seaside districts were separated from inland districts by an impassable barrier. And it may be that the reason for the Little Weighton indices being lower than those of the Bainton and Holderness Inland districts is that those two districts receive more support from neighbouring earlier sowing districts.

One fact, though it scarcely requires census work to demonstrate it, is brought out quite clearly by a comparison of the figures for the different broods. One female fly contains about a hundred eggs, but the charts show that the Midge made but slow progress both in 1913 and 1914. There must therefore be a great mortality at some stage or stages of the life-history even in fine weather. This is doubtless due to a variety of causes.

In seaside districts, I believe, many flies are lost in the sea, but inland probably a large number of flies fail to find swedes—perhaps fail to find a mate—and die without leaving offspring.

There is probably a considerable mortality in the soil. In experiments I have only reared about 30 % of the maggots put into soil, even with plenty of moisture. And maggots die if they get too dry. In one experiment 100 maggots were put into dry soil from the surface of a garden bed and kept at a temperature at which maggots with plenty of moisture will pupate successfully. In this experiment no flies appeared and on searching the soil four months later I found 99 dead maggots. Lack of moisture in the soil is therefore a probable source of loss.

The hoeing up in singling of plants harbouring small maggots must surely be a source of loss, though maggots are able to extricate themselves from plants left lying on soil, and quite small maggots are able to pupate successfully, though, of course, they turn into small flies. But I find that the proportion of small maggots which pupate successfully is lower than the proportion of larger maggots, and that such small flies contain fewer eggs than larger flies although the eggs in both are about the same size.

The Midge has some enemies. An Empid fly which sucks the juices from the flies was common at Garforth in June 1914. Some Midges are accounted for by small spiders. And in September 1914 in some experiments I reared a number of Proctotrypid flies from Swede Midge maggots.

In 1912 in the East Riding the competition of the Turnip Flea Beetle, *Phyllotreta nemorum*, was a matter of some importance. The Flea Beetle attacks the seed leaves of swede plants while the Midge does not attack them until they get into rough leaf. In 1912 the Flea Beetle was rampant in the East Riding and often made it necessary to sow two or even three times.

One more conclusion may be drawn from a comparison of the figures for the different years. If it is not already possible it probably would be possible after a few years further work to foretell, roughly, before the first brood makes its appearance, the sort of attack which may be expected. In 1911 the Midge increased enormously in number and the first brood of 1912 was a very large one. In 1912 the numbers of the Midge were greatly reduced and the first brood of 1913 was a very small one. In 1913 the Midge increased in number, but did not make much progress, and the first brood of 1914 was by no means numerous. If it is the weather which is the chief factor responsible for these ups and downs of the Midge, then weather statistics alone for a summer, without actual observations in the field, would probably be enough to enable one to make a more or less accurate prophecy of what sort of attack to expect. Unless winter weather is of much importance, and there has not been a severe winter since I first became interested in the Midge, it would be possible to make this prophecy six or eight months before the first brood appeared. I have already pointed out (p. 96), however, the possible complication which may be introduced by parasites. As I have already remarked, it may be that the relation of the Midge to the weather is not so simple as it may at present appear to be.

VI. SUMMARY OF CHIEF CONCLUSIONS.

1. The Swede Midge was found to be present in 1912, 1913, and 1914 both at Garforth in the West Riding of Yorkshire and in all parts of the area in which I worked in the East Riding. At the same time it was possible to measure differences, sometimes very marked, in the relative numbers of the Midge at different places and at different times.

2. Conditions determining the percentage of plants attacked by Brood I of the Midge in a given field are:

(1) Date of sowing (p. 89),
(2) Distance from a swede field of the previous year, and
(3) Size of field (p. 89).

3. In an early sown swede field adjacent to an early sown swede field of the previous year a drop, often a very marked one, in the percentage of attacked plants could usually be plotted as the distance in that field from the swede field of the previous year increased (pp. 89, 90).

4. An appreciable number of flies of broods later than the first sometimes leave the field in which they have spent the larval and pupal periods, and lay their eggs in another field (p. 91).

5. Factors determining the Swede Midge index in a given district seem to be:

(1) Average date of sowing (p. 92).
(2) Distance from the sea (p. 94).

6. The prosperity of the Swede Midge appears to depend very largely on summer weather (p. 95). In this connection, however, the relation between the Midge and any parasites requires investigation. I have reared Proctotrypid flies from larvae of the Midge (p. 98).

7. Very likely the Midge would cease to exist in the Holderness Coast district if it were not for the immigration of flies into that district.

8. There is a great mortality at some stage or stages of the life-history.

9. In 1912 in the East Riding the Turnip Flea Beetle, *Phyllotreta nemorum*, was a competitor of some importance with the Midge.

APPENDIX.

1913 and 1914. Dates of surveys and tests of accuracy of surveys.

1913 *Brood I.*

July 9—31.
One field at Driffield repeated.
Survey, July 16 and 18, in two rows, 354 infected plants.
Repeated, Aug. 6, in same two rows, 374 infected plants.

1913 *Brood II.*

Aug. 12—Sept. 12.
Six fields repeated in Districts III and IV.
Survey, Aug. 14—20, % CL, 0·25, 2·5, 0·7, 4·5, 3·5, 1·0.
Repeated, Sept. 9, fields in same order, % CL, 0·25, 2·5, 1·25, 5·0. 4·0, 2·0.
This I believe to be the least accurate of my five surveys.

1914 *Brood I.*

July 7—26.
Two fields repeated.
A, in District VII. Survey, July 10, in three rows, 241 infected plants.
Repeated July 26, in same three rows, 214 infected plants.
B, in District IX. Survey proper, July 15, in two rows, 95 infected plants.
Repeated, July 26, in same two rows, 95 infected plants.

1914 *Brood II.*

Aug. 17—28.
Seven fields repeated in District V.
Survey, Aug. 18, % CL, 6, 9, 10, 14, 13, 10, 8.
Repeated, Aug. 28 and 29, fields in same order, % CL, 9, 12, 10, 14, 8, 11, 9.

1914 *Brood III.*

Sept. 22—Oct. 4.
Eight fields repeated in District V.
Survey, Sept. 22, % CL, 23, 15, 15, 21, 12, 19, 13, 16.
Repeated, Oct. 4, fields in same order, in same rows as in survey proper, % CL, 18, 21, 13, 19, 10, 18, 13, 13.

There was no danger of more than very slight inaccuracy in each of the Brood I surveys from more Brood I flies coming out after the survey began, for each year the Brood I maggots had to a large extent left the plants before the Brood I survey began.

In the Brood II test fields, in both years, neighbouring rows to those examined in the survey were selected, not the actual rows examined in the survey, because in examining a plant at the time when infected leaves were small before deciding whether it was attacked or not I had sometimes destroyed the signs of attack. In the other surveys I was able to avoid doing this in the fields which I afterwards repeated as tests.

TABLE 1.

Meteorological data for the period June 1st to Sept. 30th for each of the years 1911, 1912, 1913, 1914, *and average percentages of plants attacked by the Swede Midge* (% *CL*). *The figures given below are calculated from figures published in the monthly weather reports of the Meteorological office. The figures given for the East Riding are the mean of figures calculated for Scarborough and Hull.*

	Year	Average Mean Air Temperature, °F.	Bright sunshine, hours	Rainfall, inches	Number of days on which rain fell (out of 122 days)	Average % CL (Brood I)	Average % CL (Brood II)	Average % CL (Brood III)
East Riding	1911	60·2	793	10·58	51	—	—	—
	1912	56·2	335	13·72	73	38*	—	—
	1913	57·7	520	3·40	40	1·2	2·4	—
	1914	58·7	610	9·73	44	2·3	3·2	6·6
Garforth	1911	58·2	783	8·26	46	—	—	—
	1912	55·4	380	14·16	67	94†	—	—
	1913	56·3	512	4·05	50	1·9	—	—
	1914	57·1	688	8·77	47	22	32	76

* 27 fields were examined between July 16th and 29th.

† 7 fields were examined on August 2nd.

TABLE 2.

1913. *Average percentages of attacked plants and Swede Midge indices for each district for the two surveys. The averages are given for May sown fields, June sown fields, and for all fields irrespective of sowing date.*

		Brood I							Brood II							
		May sown		June sown		All fields			May sown		June sown		All fields			
	District	Number of fields	Average % CL	Number of fields	Average % CL	Number of fields	Average % CL	Swede Midge index	Number of fields	Average % CL	Number of fields	Average % CL	Number of fields	Average % CL	Swede Midge index	Increase or not over Brood I index
I.	Filey	8	1·7	3	1·4	11	1·7	2	8	3·1	3	2·8	11	3·0	3	Yes
II.	Bempton........	9	2·4	2	0·2	11	2·0	2	9	2·8	2	0·7	11	2·4	3	Yes
III.	Flamborough ...	10	0·8	1	0·2	11	0·7	1	10	0·4	1	0·2	11	0·4	1	No
IV.	Dale Towns	3	2·2	8	0·5	11	0·9	1	3	2·7	8	2·3	11	2·4	3	Yes
V.	High Wolds	14	0·9	6	0·1	20	0·7	1	14	3·6	6	2·7	20	3·3	4	Yes
VI.	Reighton........	8	3·3	3	0·5	11	2·5	3	8	4·5	3	3·0	11	4·1	5	Yes
VII.	Low Wolds	11	2·8	16	0·5	27	1·5	2	11	5·8	16	5·0	27	5·5	6	Yes
VIII.	Bainton	—	—	4	1·7	4	1·7	2	—	—	4	1·6	4	1·6	2	No
IX.	Holderness Inland	7	1·3	10	0·2	17	0·7	1	7	2·6	10	2·4	17	2·5	3	Yes
X.	Holderness Coast.	4	1·3	16	0·1	20	0·4	1	4	0·5	16	0·5	20	0·5	1	No
XI.	Little Weighton..	5	0·5	3	0·1	8	0·4	1	5	0·7	3	0·1	8	0·5	1	No
	Averages (East Riding)	—	1·7	—	0·5	—	1·2	—	—	2·7	—	1·9	—	2·4	—	—
XII.	Garforth	12	1·9	—	—	12	1·9	2	—	—	—	—	—	—	?	—

TABLE 3.

1914. *Average percentages of attacked plants and Swede Midge indices for each district for the three surveys. The averages are given for May sown fields, June sown fields, and for all fields irrespective of sowing date.*

	Brood I							Brood II								Brood III							
	May sown		June sown		All fields			May sown		June sown		All fields				May sown		June sown		All fields			
District	Number of fields	Average % CL	Number of fields	Average % CL	Number of fields	Average % CL	Swede Midge index	Number of fields	Average % CL	Number of fields	Average % CL	Number of fields	Average % CL	Swede Midge index	Increase or not over Brood I index	Number of fields	Average % CL	Number of fields	Average % CL	Number of fields	Average % CL	Swede Midge index	Increase or not over Brood II index
I. Filey	11	4·0	1	1·0	12	3·7	4	11	3·8	1	1·0	12	3·6	4	No	11	7·1	1	7·0	12	7·1	8	Yes
II. Bempton	8	3·8	—	—	8	3·8	4	8	3·5	—	—	8	3·5	4	No	8	7·3	—	—	8	7·3	8	Yes
III. Flambro'	9	0·6	2	0·5	11	0·5	1	9	1·2	2	2·0	11	1·2	2	Yes	9	1·9	2	1·0	11	1·7	2	No
IV. Dale Towns	10	3·6	5	2·6	15	3·3	4	10	5·3	5	4·4	15	5·0	5	Yes	10	17·2	5	8·9	15	14·4	15	Yes
V. High Wolds	21	3·0	3	1·0	24	2·7	3	21	5·9	3	1·7	24	5·4	6	Yes	21	12·0	3	4·0	24	11·0	11	Yes
VI. Reighton	10	4·6	—	—	10	4·6	5	10	5·8	—	—	10	5·8	6	Yes	10	13·0	—	—	10	13·0	13	Yes
VII. Low Wolds	19	3·5	8	1·2	27	2·8	3	19	4·8	8	2·3	27	4·0	4	Yes	19	8·5	8	2·7	27	6·8	7	Yes
VIII. Bainton	5	1·7	4	1·6	9	1·7	2	5	3·3	4	2·8	9	3·1	4	Yes	5	6·1	4	3·3	9	4·8	5	Yes
IX. Holderness Inland	21	1·9	23	1·1	44	1·5	2	21	2·9	23	2·0	44	2·4	3	Yes	21	6·3	23	2·8	44	4·5	5	Yes
X. Holderness Coast	7	0·8	14	0·5	21	0·6	1	7	0·8	14	0·4	21	0·6	1	No	7	0·5	14	0·1	21	0·3	1	No
XI. Little Weighton	3	0·5	5	0·3	8	0·4	1	3	0·7	5	1·0	8	0·9	1	No	3	1·0	5	1·3	8	1·2	2	Yes
Averages (East Riding)	—	2·5	—	1·1	—	2·3	—	—	3·5	—	2·0	—	3·2	—	—	—	7·4	—	3·5	—	6·6	—	—
XII. Garforth	12	22	—	—	12	22	22	12	32	—	—	12	32	32	Yes	12	76	—	—	12	76	76	Yes

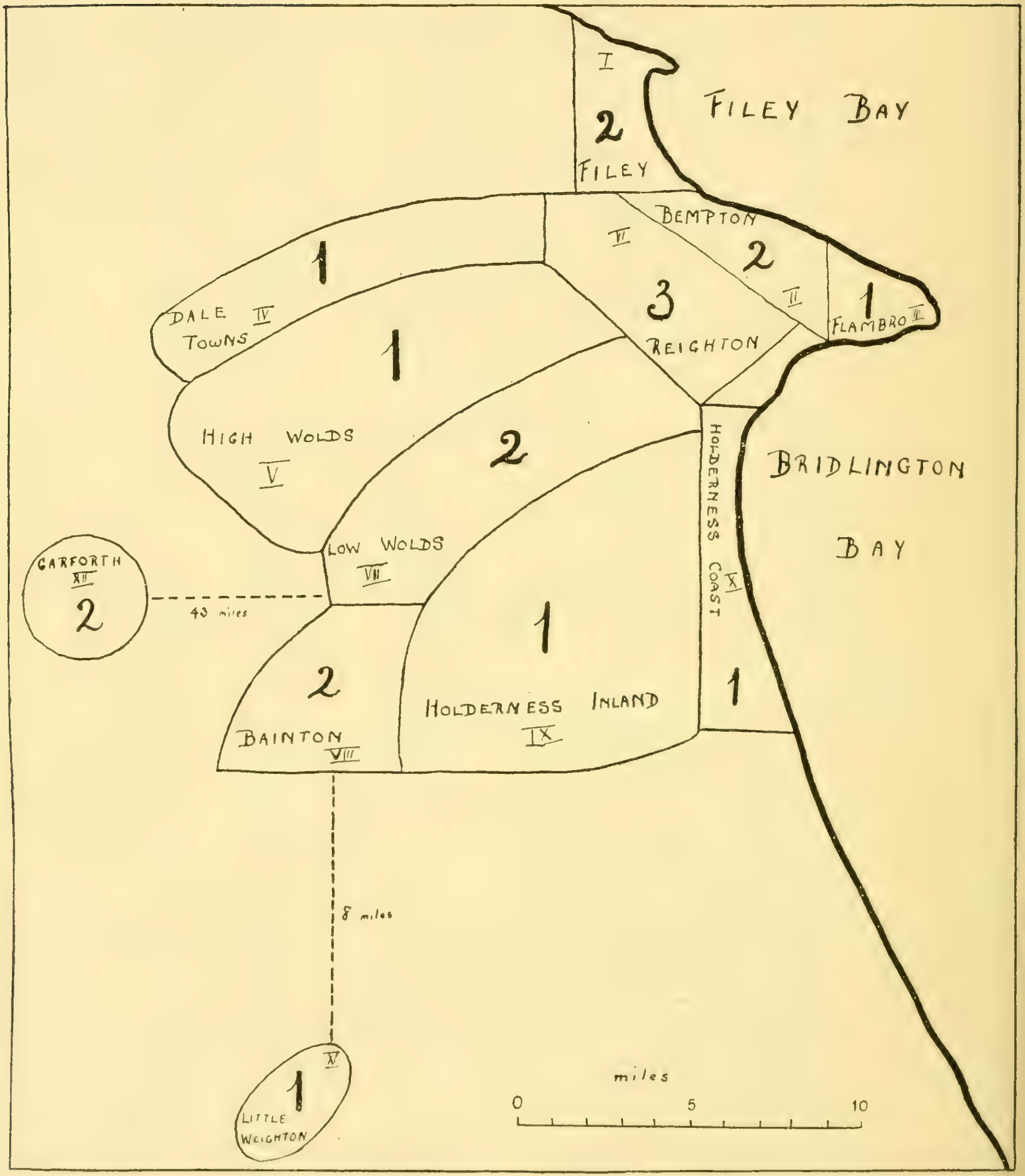
I
2
FILEY
FILEY BAY
BEMPTON
VI
2
II
1
FLAMBRO II
1
DALE TOWNS IV
1
3
REIGHTON
HIGH WOLDS
V
2
HOLDERNESS COAST X
BRIDLINGTON BAY
CARFORTH XII
2
43 miles
LOW WOLDS
VII
1
2
BAINTON
VIII
HOLDERNESS INLAND
IX
1
8 miles
XI
1
LITTLE WEIGHTON
miles
0
5
10

Chart 1. Showing for each district the relative density of Brood I of the Swede Midge in 1913.
Roman numeral = number of district.
Arabic numeral = index (Brood I).

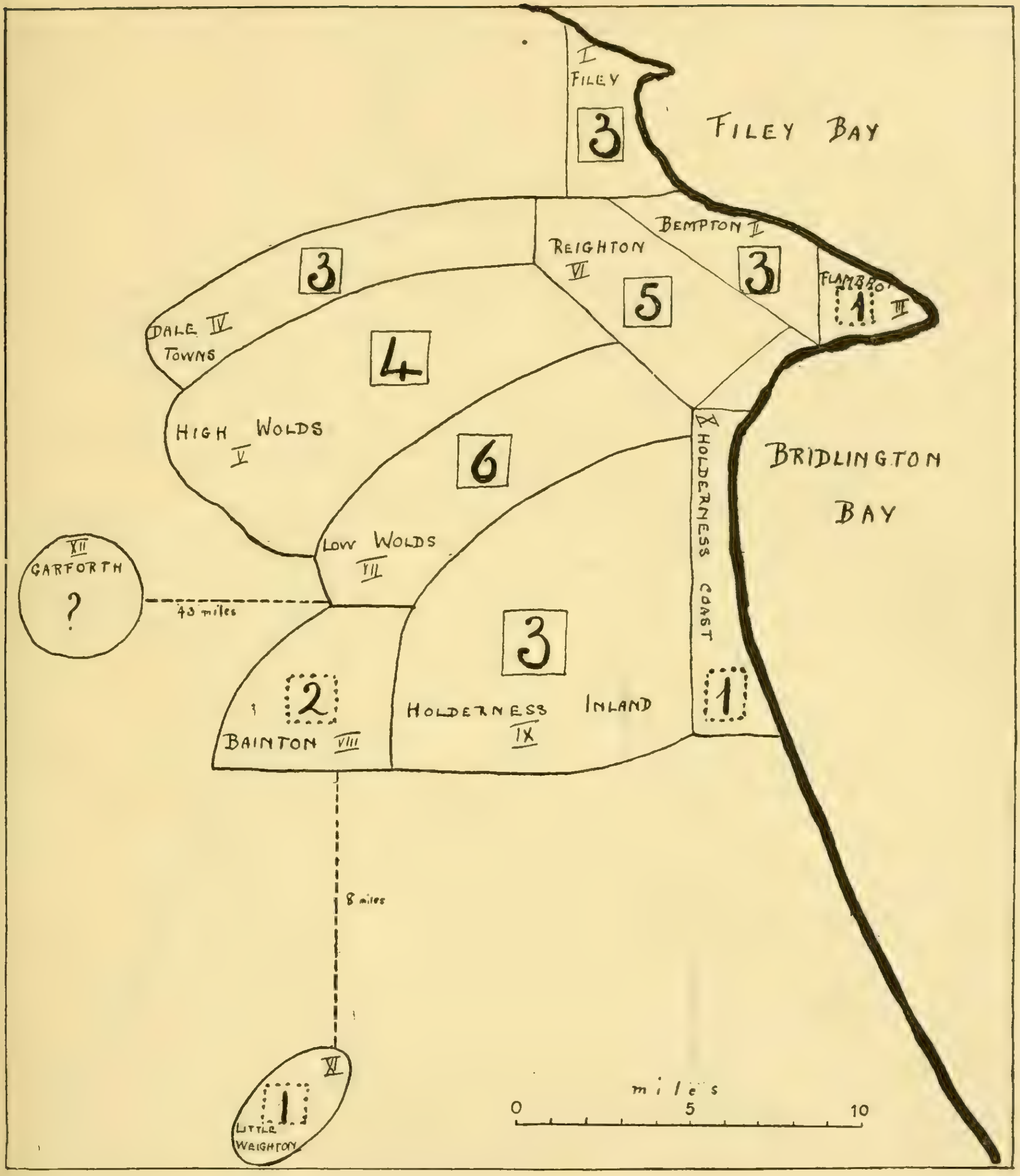
I
Filey
3
Filey Bay
Bempton II
3
Reighton
VI
5
Flambro'
1
III
3
Dale IV
Towns
4
High Wolds
V
6
X Holderness Coast
Bridlington
Bay
XII
Garforth
?
43 miles
Low Wolds
VII
2
Bainton VIII
3
Holderness Inland
IX
1
8 miles
XI
1
Little
Weighton
miles
0
5
10

Chart 2. Showing for each district the relative density of Brood II of the Swede Midge in 1913.
Roman numeral = number of district.
Arabic numeral = index (Brood II).

A rectangle of unbroken lines round the index figure indicates that the Brood II index exceeds the Brood I index.

A rectangle of dotted lines round the index figure indicates that the Brood II index does not exceed the Brood I index.

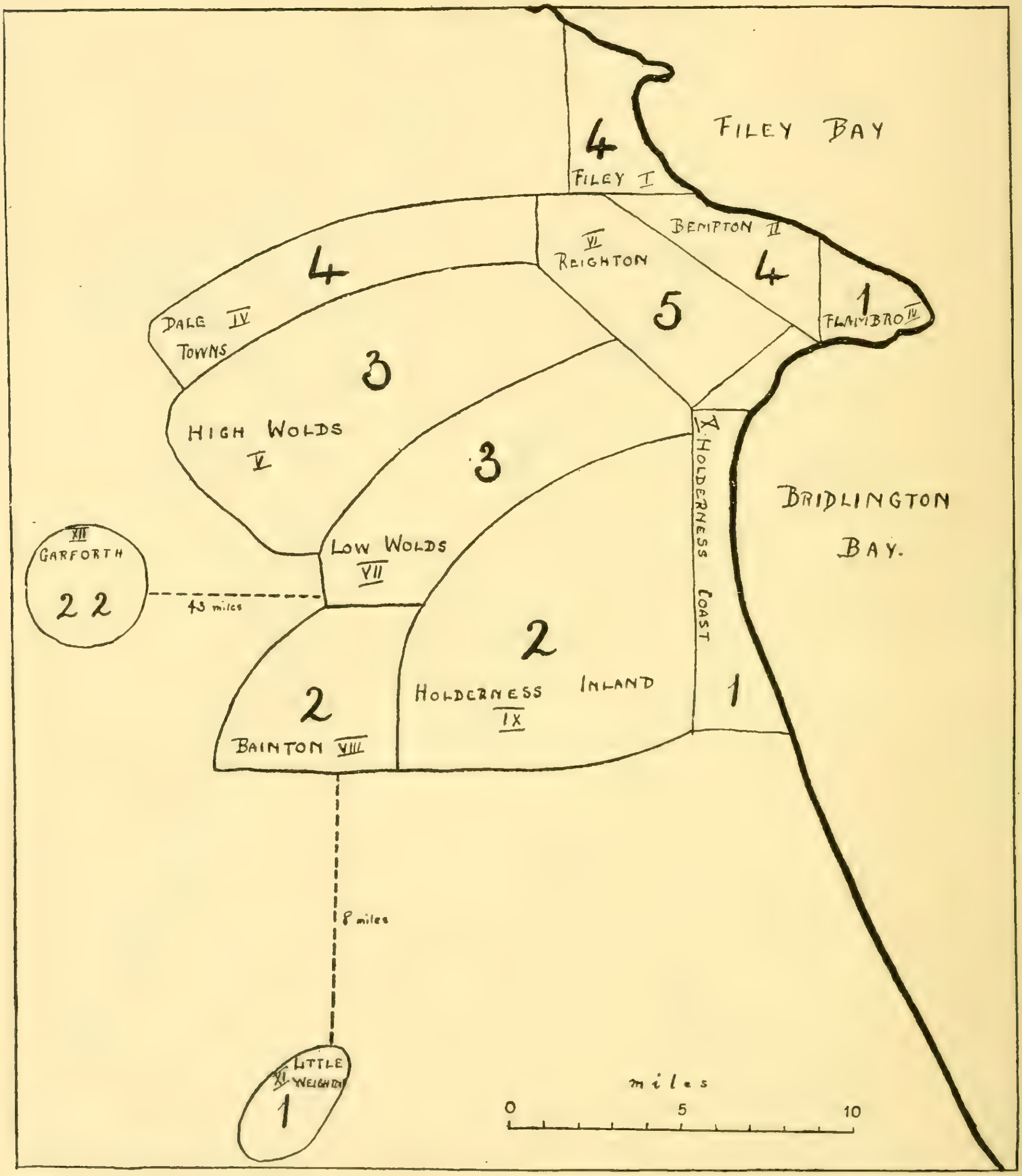
FILEY BAY
4
FILEY I
BEMPTON II
4
VI REIGHTON
5
1
FLAMBRO IV
4
DALE IV TOWNS
3
HIGH WOLDS V
3
LOW WOLDS VII
BRIDLINGTON BAY.
X HOLDERNESS COAST
1
XII GARFORTH
22
43 miles
2
BAINTON VIII
2
HOLDERNESS INLAND IX
8 miles
XI LITTLE WEIGHTON
1
miles
0
5
10

Chart 3. Showing for each district the relative density of Brood I of the Swede Midge in 1914.
Roman numeral = number of district.
Arabic numeral = index (Brood I).

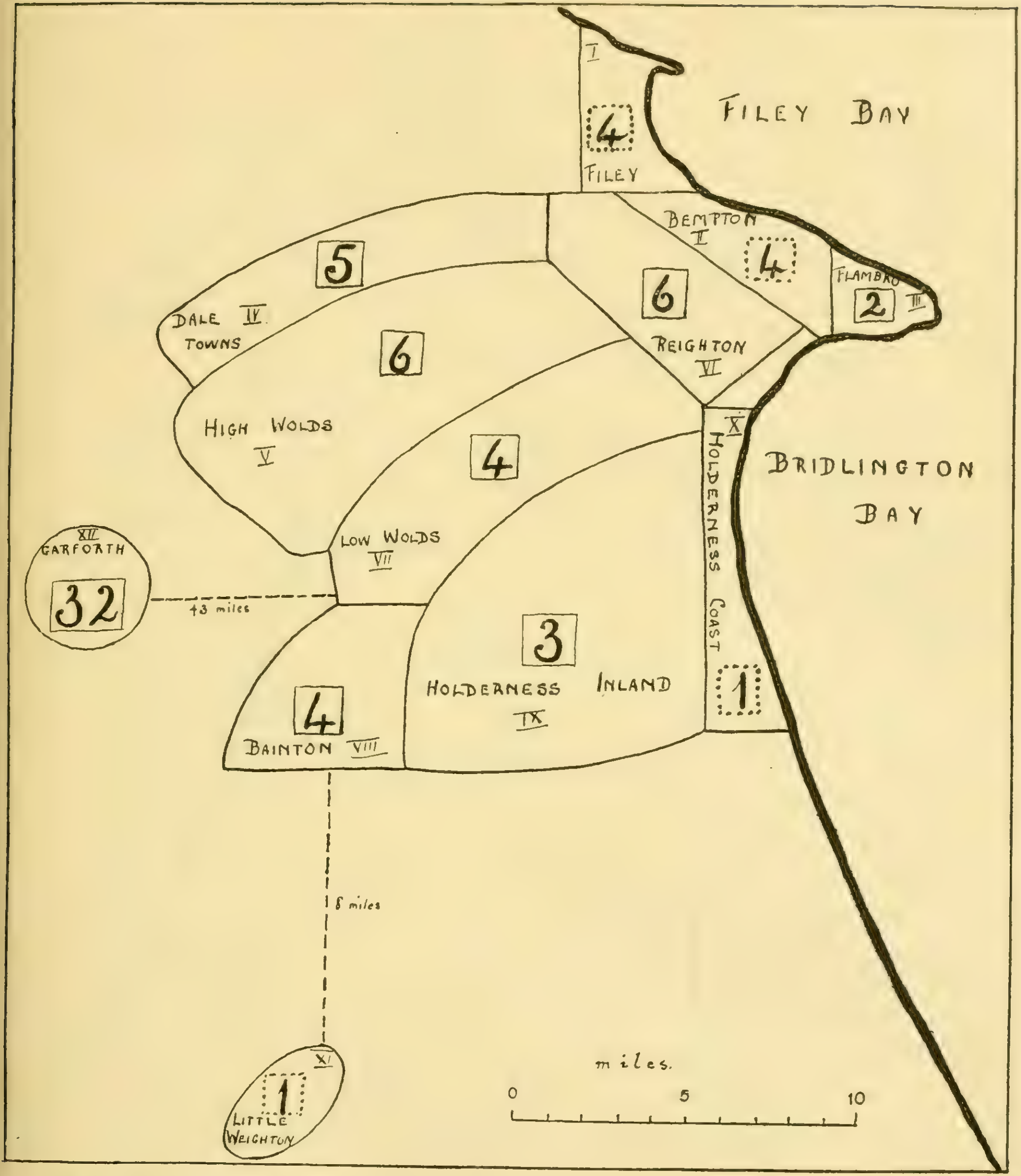
I
4
FILEY
FILEY BAY
BEMPTON
II
4
FLAMBRO
2
III
5
DALE TOWNS
IV
6
6
REIGHTON
VI
HIGH WOLDS
V
4
X
HOLDERNESS COAST
BRIDLINGTON BAY
XII
CARFORTH
32
LOW WOLDS
VII
43 miles
3
HOLDERNESS INLAND
IX
1
4
BAINTON VIII
8 miles
XI
1
LITTLE WEIGHTON
miles.
0
5
10

Chart 4. Showing for each district the relative density of Brood II of the Swede Midge in 1914.
Roman numeral = number of district.
Arabic numeral = index (Brood II).

A rectangle of unbroken lines round the index figure indicates that the Brood II index exceeds the Brood I index.

A rectangle of dotted lines round the index figure indicates that the Brood II index does not exceed the Brood I index.

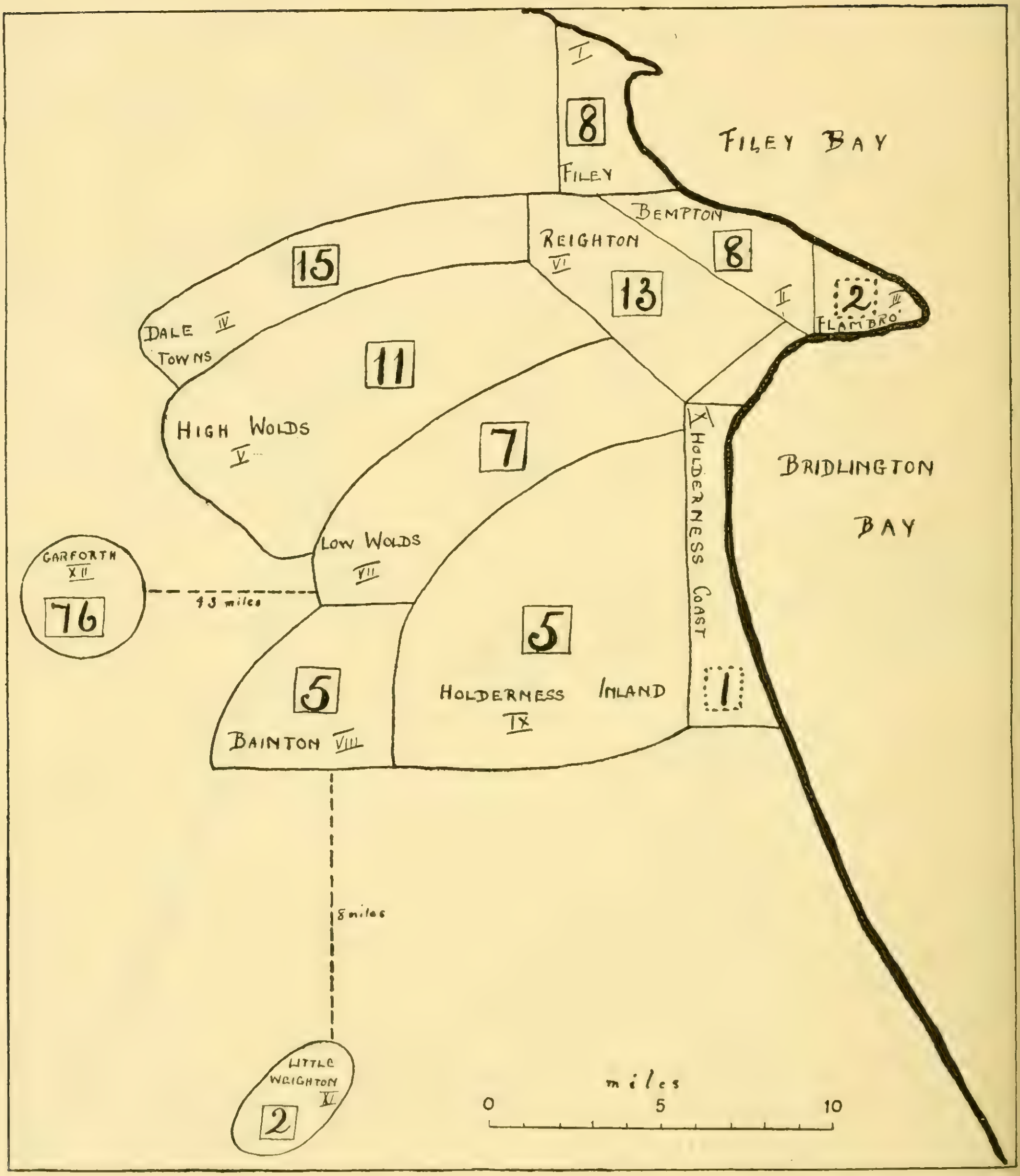
I
8
FILEY
FILEY BAY
BEMPTON
8
II
2
III
FLAMBRO'
REIGHTON
VI
13
15
DALE IV
TOWNS
11
HIGH WOLDS
V
7
LOW WOLDS
VII
X
HOLDERNESS COAST
BRIDLINGTON
BAY
GARFORTH
XII
76
13 miles
5
HOLDERNESS INLAND
IX
1
5
BAINTON VIII
8 miles
LITTLE
WEIGHTON
XI
2
miles
0
5
10

Chart 5. Showing for each district the relative density of Brood III of the Swede Midge in 1914.
Roman numeral = number of district.
Arabic numeral = index (Brood III).

A rectangle of unbroken lines round the index figure indicates that the Brood III index exceeds the Brood II index.

A rectangle of dotted lines round the index figure indicates that the Brood III index does not exceed the Brood II index.

THE OCCURRENCE OF FUNGI ON *ALEURODES VAPORARIORUM* IN BRITAIN.

By A. S. HORNE,

Royal Horticultural Society's Gardens, Wisley.

In 1902 H. M. Lefroy[1] reported the presence of a fungus on the brown shield scale (*Saissetia hemisphaerica*) in the West Indies which was subsequently identified as *Cephalosporium Lecanii* discovered by Zimmermann[2] infesting *Lecanium viride* (green bug) on the coffee plant in Java (1898). Both Zimmermann, and Parkin[3] who described *C. Lecanii* in greater detail (1906), ascribe to the fungus a parasitic habit. It was enrolled among the ranks of entomogenous fungi and has been used on a considerable scale to combat scale insects in the West Indies, Florida and elsewhere, and according to J. R. Bovell[4], F. W. South[5], G. G. Auchinleck[6] (citrus trees in Dominica and Grenada), G. E. Bodkin[7] (British Guiana), and W. Nowell[8] (Grenada, Dominica, Antigua) with some success.

Professor Lefroy in December, 1914, drew my attention to a considerable quantity of fungus on the leaves of Centropogon, at Wisley, also badly infested, for experimental purposes, with the nymph-form of *Aleurodes vaporariorum* (white fly). The upper surface of the leaves was covered with a sooty mould (*Capnodium*?): the under surface, infested with a quantity of eggs, nymphs and a few emerging and complete imagos, was covered with mycelial tufts of *Cladosporium* and wefts of *Cephalosporium*.

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[1] H. M. Lefroy in *W. I. Bull.* III. (1902), 240, 295.

[2] Zimmermann, *Over eene Schimmel epidemic der Groene Luizen.* Buitenzorg, Java, 1898.

[3] J. Parkin in *Ann. Roy. Bot. Gard. Peradeniya,* III. Pt. 1 (1906), 41.

[4] J. R. Bovell in *W. I. Bull.* XII. No. 4 (1912), 399–402.

[5] F. W. South in *W. I. Bull.* XI. No. 1 (1911), 1–30, and *W. I. Bull.* XII. No. 2 (1912).

[6] G. G. Auchinleck in *W. I. Bull.* XII. No. 2 (1912).

[7] G. E. Bodkin in *Jour. Bd. Agric. Brit. Guiana,* V. No. 2 (Oct. 1911).

[8] W. Nowell in *W. I. Bull.* XIV. No. 3 (1914), 215.

The mode of occurrence of the *Cephalosporium* on the *Aleurodes* nymph agrees in every respect with Parkin's description and figure of *Cephalosporium Lecanii* on Lecanium, where the mycelial threads with their short capitate conidiophores are depicted radiating outwards from the body of the nymph, but in this case dead imagos invested by a weft of mycelium are also found.

Since this Cephalosporium, which will be described in detail elsewhere, proves to be quite distinct from Zimmermann's species and indeed from all other Cephalosporia, it has been named *Cephalosporium Lefroyi* (see *Gard. Chron.* March 13, 1915, p. 139).

Ever since P. H. Rolfs[1] demonstrated by orchard introductions that *Sphaerostilbe coccophila*, discovered by the Tulasnes in 1865, could be used to combat the San José scale, the use of entomogenous fungi has been extensively adopted in Florida where the conditions of temperature and moisture are conducive to the spread of fungi. Thus a list of six fungi parasitic upon *Aleurodes citri*—*Aschersonia aleyrodis* Webber, *Aschersonia flavo-citrina* P. Henn., *Verticillium heterocladum* Penz., *Sphaerostilbe coccophila* Tul., *Microcera* sp., and a sterile brown fungus, afterwards styled *Aegerita Webberi* (see *Mycologia*, II. No. 4, July, 1910)—is given by Howard S. Fawcett[2] (1908) of which *Sphaerostilbe coccophila* is stated to be rarely parasitic on this insect: a seventh is added by E. W. Berger[3] (1909), a species of *Sporotrichum*, which is stated to infect the adult and some larvae.

According to the Report of the Entomologist[4], Florida (1913), natural mortality of white fly is caused mostly by *Microcera*. On the other hand, Morrill and Back (1912)[5] report that mortality from unexplained causes proves to be the most important element of natural control. This mortality has never been taken into consideration in previous publications, and in the past no little confusion has existed owing to the failure to distinguish between the benefits derived from it and those from fungus parasites. *Microcera* is stated to be normally saprophytic.

We are therefore led to wonder to what extent the mortality amongst scale insects is really due to fungi. Both in Florida and the West

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[1] P. H. Rolfs in *Fla. Agr. Exp. Sta. Bull.* 41 (1897).

[2] Howard S. Fawcett, *Fungi parasitic upon* Aleyrodes citri. Thesis Univ. State of Florida, June, 1908.

[3] E. W. Berger in *Fla. Agr. Exp. Sta. Bull.* 97 (Feb. 1909).

[4] Florida Agr. Exp. Sta. *Ann. Rept.* (1913), LIV–LVIII.

[5] A. W. Morrill and E. A. Back in U.S.A. Dept. Agr. Bur. of Ent. *Bull.* 102 (1912)

Indies the climatic conditions which favour the development of the fungi would appear to be inimical to the insects, as in Dominica; but where and when dry conditions exist at certain times of the year, as at St Kitts, few fungus enemies to insects are found.

Some observations made in the case of *Cephalosporium Lefroyi* on *Aleurodes vaporariorum* seem to be in agreement with the conclusions of Morrill and Back. They are as follows:

1. Dead imagos occur enclosed in a weft of Cephalosporium.
2. Feeble but not always old imagos are not in every case associated with Cephalosporium.
3. Crushed feeble imagos have not always yielded hyphae and spores.
4. Feeble imagos may bear portions of the mycelium of the fungus.
5. Dead, half-emerged imagos are often coloured orange and may or may not be associated with Cephalosporium.
6. There are large numbers of dead nymphs, some with Cephalosporium, some with Cladosporium, others without fungi.

We must carefully distinguish the case of the nymphal Aleurodes from that of the imago. The nymph practically remains *in situ* on the leaf. Fungi could live and grow on the honey-dew excretion caused by the insect and ultimately invest the nymph and close the tracheae, a method of action suggested by Giard[1] for *Cladosporium parasiticum* on *Polyphylla fullo*, and which may well happen in the case of the fungi under discussion.

Clearly the question of the parasitism of *Cephalosporium Lefroyi* and the other species of fungi associated with *Aleurodes vaporariorum* must remain open until the whole subject of fungi in relation to scale insects has been reinvestigated by improved methods and put on a more satisfactory basis.

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[1] Giard in *Comptes Rendu*, CXII. 1518–21.

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THE SOUTH AFRICAN MULBERRY BLIGHT *BACTERIUM MORI* (BOY. AND LAMB.) SMITH.

BY ETHEL M. DOIDGE, M.A., F.L.S.

Mycologist, Division of Botany, Pretoria.

(With Plates XIX–XXIV.)

FOR some years it has been evident that the finer varieties of the mulberry, particularly the one known as the "English mulberry," do not thrive in certain districts of the Union. I have seen trees of this variety 14 years old which have only attained to a height of three to five feet. In other districts the black mulberry grows well, and fine trees may be seen, so that the failure cannot be attributed to unsuitability of climate; the readiness with which the common mulberry grows would also go to disprove such a supposition. The general appearance of the trees suggests that they are suffering from some blight: a number of the tips of the branches are dead, and the leaves towards the end of the season become covered with small brown spots.

In November, 1908, leaves and twigs so affected were sent in for examination from a farm in the Pretoria district and the diseased areas were found to be occupied by countless swarms of bacteria. No fungus was found in connection with the disease, so that the bacillus was probably the causal organism, and culture work was undertaken in order to discover whether or not the South African mulberry blight is identical with that known in Europe and America.

The literature on the mulberry blight shows that the disease has been attributed to two entirely different bacilli, and it therefore becomes necessary to briefly review such literature in order to compare the characters ascribed to these organisms.

Literature.

In Italy the mulberry blight has received considerable attention owing to its supposed connection with the disease of silkworms known as "flâcherie." The work of the Italian writers will be considered first.

The blight on the leaves was first studied in 1890 by Cuboni and Garbini (2), who attributed the trouble to a Diplococcus. They claimed to have isolated the organism in pure culture, and with it to have reproduced the disease in healthy trees. They held that this Diplococcus was related to, though not identical with, *Streptococcus bombycis* (Flügge) the causal organism of "flâcherie" and found that it was pathogenic to silkworms.

Macchiati (3, 4, 5) working in the years 1891–2 also found the bacillus parasitic on the mulberry pathogenic to silkworms but quite distinct from *Streptococcus bombycis.* He studied the organism more fully and named it *Bacillus cubonianus.*

Voglino (10) published a paper on the bacteriosis of the mulberry in 1894. He isolated from affected tissues an organism pathogenic to silkworms; however, his description of this organism does not agree with that of *B. cubonianus* and his results have not been confirmed by more recent workers.

The most recent of the Italian writers is Peglion (7), who in 1897 confirmed Macchiati's work and gave an account of the disease and the organism causing it. A brief résumé of this paper follows in order that the characters which he assigns to *B. cubonianus* may be compared with those of *Bacterium mori* to which the disease has been attributed by French and American writers.

The first traces of disease are found on the leaves, a slight discoloration of the parenchyma being noticeable at certain points, especially if the leaf is held up to the light. Many of the spots affect the veins, then the leaves become curled and wrinkled, and in any case they become torn and finally reduced to tatters. The young shoots are also attacked; first of all projecting blisters are formed, bright brown in colour, later they become dull and sunken. The first apical internode, where the tissues are tender, is usually the one attacked. The infected area extends in the form of a streak 3–4 cm. long; if only one side of the shoot is affected it causes curvature, but frequently the affected area extends all round the shoot, in which case the extremity wilts and falls.

From such tissues a yellow bacillus was isolated, which rapidly liquefies gelatine, and grows well on agar and potato, becoming intensely yellow. It does not exceed 2μ in length, the average being $1\frac{1}{2}\mu$; chains were observed in gelatine cultures. With cultures of this organism suspended in distilled water, characteristic infections were obtained in three days. The experiments, however, were made with detached leaves and shoots kept moist under a bell jar, and there is no record of any controls having been kept.

A study was also made of the anatomical characters of the diseased tissues. In the shoots the cortical tissues are first attacked; infection then progresses in a radial direction as far as the cambium, and sometimes the wood is affected, the latter only in cases where infection takes place before the tissues are properly differentiated. In the infected tissues the cell contents slowly disintegrate and become transformed into a brown substance which is not stained by ordinary aniline dyes. The death of cells in the infected areas and the continued growth of the surrounding parts lead to the formation of small longitudinal cracks; these are frequently filled up the following year by the formation of scar tissue.

In 1894, Boyer and Lambert (2) in France described a bacterial blight on the leaves and shoots of the mulberry, the external effects of which were very similar to those of the Italian blight. They isolated an organism from the diseased tissues and with cultures of this they reproduced the disease on healthy bushes. They named their organism *Bacterium mori* but did not describe it.

A paper published in *Science* in 1910 gave the results obtained by Erw. F. Smith (8), who studied the disease in America. He was unable to obtain infections with any yellow organisms which liquefied gelatine but isolated a white organism which was markedly pathogenic. He obtained numerous infections with pure cultures of this organism and so also did several of his co-workers, working independently. The organism and its effect on the host plant were described in detail, and the name *Bacterium mori* was retained. As this organism appears to be identical with the one isolated from blighted mulberry shoots in this country, a detailed account of the bacterium is given elsewhere. Smith concluded that either there are two organisms (*Bacillus cubonianus* and *Bacterium mori*) capable of causing a blight of the mulberry, or that the Italian workers secured inoculations with mixed cultures.

In 1914, Smith (9) published a further note stating that he was convinced of the identity of the French and American blights, having seen when in Paris, specimens of blighted mulberry twigs collected in

France. He was also shown a culture of a white organism and twigs and leaves blighted by it, which had been inoculated some six weeks previously. The signs, internal and external micro- and macroscopic, on the tree agreed perfectly with the American disease and the streaks looked exactly the same.

Infected shoots and leaves were sent to Washington; from these *Bacterium mori* was isolated and successful inoculations made. In view of the fact that both American and French diseases are certainly caused by *B. mori*, Smith considers that the Italian disease should be re-examined; all the external signs of the disease being identical in both countries.

Geographical Distribution in South Africa.

The mulberry blight is very severe in the Pretoria district; I have not seen a single black mulberry (*Morus nigra*) which has escaped infection. Not only are trees in the town and its neighbourhood affected but in such farms and outlying places as Garstfontein and Onderstepoort.

It is pretty general in Natal, and specimens have been received from Bloemfontein, O. F. S., and Pietersburg, Transvaal, very badly infected with the blight.

Mr R. A. Davis, the Government Horticulturist, informs me that he has never seen the disease in the Western Province of the Cape, and that there one finds very fine specimens of the black mulberry tree. Possibly the winter rains and dry summers are partly accountable for the immunity of the trees in this region, as the new infections on leaves and shoots are only observable in the Transvaal after the first spring rains.

The common mulberry never becomes conspicuously blighted in nature although it is possible to infect its leaves and shoots with pure cultures of the causal organism.

At Mr Davis's suggestion we obtained specimens of leaves of several varieties of mulberry grown by Mrs Forbes of Athol in the Ermelo district. Of these, *M. nigra* and *M. alba* were found to be infected and also another species which has not been identified. This is the only record of varieties other than *Morus nigra* being found infected with the blight.

External Characteristics of Disease.

The appearance of leaves attacked by the bacterial blight is quite distinct from that of leaves attacked by the common leaf spot fungus (*Septogloeum mori* Bri. and Cav.); the spots being smaller, darker in colour and more numerous.

The first indication of infection is the appearance on the under side of the leaves of very minute water-soaked areas (Plate XIX, fig. B); these increase somewhat in size though individual spots rarely exceed 2 mm. in diameter. After some days the spots begin to discolour, and gradually become dark brown or almost black. They are always angular and often become white in the centre. The attacked tissues become quite dead and later fall away leaving holes in the leaf-tissues, and the leaves assume a torn and ragged appearance (Plate XIX, fig. A); they are sometimes completely reduced to tatters. Frequently a large number of infections on a young leaf occur in the neighbourhood of a vein, in which case the vein is affected and becomes wrinkled up and growth ceases in the affected region, with the result that the leaf is distorted (Plate XX, fig. A). Badly affected leaves sometimes turn yellow and fall.

On the shoots infections are also frequent, and usually take place in rapidly growing tissues. They first appear as short, water-soaked, somewhat raised streaks, which may increase to a length of 3–4 cms. The infected portions later become sunken and discoloured (Plate XXI). If the whole of the circumference is affected the young shoot dies, and trees attacked by blight are readily detected by the numerous bare, dead twigs which they bear (Plate XX, fig. B).

This blighting of the young shoots when they appear in spring is also largely responsible for the stunted appearance of the trees, as it is only when the rains are exceptionally late and the disease consequently slow in spreading that the trees are able to make any appreciable amount of new growth.

The tissues in the affected streaks being killed and the rest of the stem continuing to grow, a tension is set up which results in the formation of longitudinal cracks in the diseased parts (Plate XXI).

Attempts at Control.

All the ordinary fungicides have been tried in order to check this disease, particularly Bordeaux mixture, but this has proved quite useless. Mr F. J. Birkett of Dundee, who has been experimenting with various methods, reports good results by using lime-sulphur as a spray and cutting away a large quantity of diseased wood during the winter. It is too soon to judge whether this treatment has been really effective, but the pruning away of diseased material is certainly a sound step, if the prunings are promptly burnt.

Infection Experiments.

From the first specimens of blighted leaves from the Pretoria district which were seen in November, 1908, a white bacterium was isolated and numerous infections were obtained on two young trees in the greenhouse by spraying them with a suspension of the culture in distilled water. At that time the work was not continued as there was other work on hand of a more pressing nature.

In September, 1913, diseased leaves were received from Pietersburg, and from these a white bacterium was again isolated. On the 25th of that month a pure culture of the organism was suspended in sterile distilled water and sprayed on a young mulberry tree (common variety) with an atomiser. Numerous minute water-soaked spots were visible on the leaves on the 30th; these became larger and by the 9th October were beginning to turn brown. Control trees sprayed with distilled water showed no trace of infection. From spots on this tree the organism was again isolated and with the second series of cultures a second tree was inoculated. The leaves were sprayed as before and the shoots pricked with a fine needle. In five days the characteristic spots developed on the leaves, and on the stems in the neighbourhood of the needle pricks a somewhat raised, water-soaked looking streak which later became swollen and discoloured.

What was apparently the same disease was observed at Bloemfontein, O. F. S., and at Dundee in Natal. Spotted leaves collected at the latter place in May, 1913, and kept dry in the laboratory yielded vigorous cultures in August, 1914. On August 21st, a young mulberry tree which was shooting out vigorously and had a number of young leaves was sprayed with a suspension of a 48 hour old agar culture. The shoots were pricked with a fine needle. Very numerous, minute, water-soaked spots were visible on the under side of the leaves on

August 27th (Plate XIX, fig. B), the young leaves showing by far the greater number. The number of spots varied from 20 to several hundred on each leaf, and many of them were in the neighbourhood of the veins. The spots did not begin to discolour for more than a week. Leaves which had been infected in the neighbourhood of the veins before the leaf had attained its maximum size were wrinkled and distorted and frequently curved over to one side (Plate XX, fig. A). After two months most of the infected areas had dried up and fallen out, leaving the leaves torn and ragged (Plate XIX, fig. A).

Infections were also obtained on the stems as described above, the affected area forming a streak 1–4 cms. long. Numerous small stem infections also occurred in parts of the stem which had not been pricked.

On August 28th the organism was again plated out from one of the recently infected leaves and a pure culture immediately obtained, and with this infections were again obtained on a young tree.

In the above experiments the common variety of the mulberry was used as no trees of the black mulberry (*Morus nigra*) could be obtained in Pretoria which were not blighted. Controls were kept in every case and these remained perfectly healthy.

Morbid Anatomy.

In addition to the fact that infection can take place through an unwounded surface and that infection is first evident on the under side of the leaf where the stomata are situated, the distribution of the bacteria in the leaf tissues also points to the probability of stomatal infection. Unfortunately, up to the present I have been unable to obtain slides showing very early stages of infection but sections through leaves bearing a few small spots still in the water-soaked stage included a large number showing the conditions depicted in Plate XXII. A dense mass of bacteria crowds the intercellular spaces near the stoma and occupies the substomatal cavity: spaces more remote from the stoma are also occupied but the bacteria in these are not so numerous. This infected area, however, was in rather close proximity to a portion of the leaf in a more advanced stage of infection, and therefore did not furnish any conclusive evidence that the bacteria had entered through the stoma as there was a possibility they had travelled through the intercellular spaces from the neighbouring infected tissues. At this stage the bacteria are entirely limited to the intercellular spaces and have not invaded the cells, which are still intact.

Later, the increasing mass of bacteria wedges the cells apart, plasmolysis takes place and the contents appear as a contracted disintegrated mass in the centre of the cell which stains deeply with carbol fuchsin. The bacteria then invade the cells themselves and complete their destruction. In this way the palisade cells and the parenchyma of the mesophyll are entirely destroyed at the points attacked. The bacteria also enter the vessels of the fibrovascular bundles.

The conditions described above are those found in infected spots on the leaves of *Morus nigra*. In this species the mesophyll is very loose in texture and the intercellular spaces large, so that the bacteria have no difficulty in penetrating these tissues. The leaf of the common variety is much thinner, the tissues more compact and the intercellular spaces correspondingly small. Sections through leaves infected by pure culture in which the water-soaked spots were just visible on the under surface showed a slightly different state of affairs (Plate XXIII). Here the bacteria have multiplied enormously in the substomatal cavity and for some distance have levered away the epidermis from the adjacent cells.

In the stem if infection takes place, as it frequently does near the tip where the tissues are tender, all parts are equally affected; if the whole of the circumference becomes involved the end of the twig dies. Frequently only one side of the stem is attacked and sometimes the infection penetrates to the pith, which shows a yellow discoloration.

In older parts of the stem the bacteria are for the most part restricted to the cortex and to the vessels of the wood. Tyloses are frequent in the latter. A fairly deep crack forms in the middle of the infected area owing to the strain on the dead cells caused by the living parts of the stem which continue to grow. Round this a cork cambium is formed and the dead cells are cut off by a layer of cork.

The dead tissues in stem and leaf assume a bright brown colour and do not stain with ordinary aniline dyes.

Morphology.

The cause of the disease is a long rod with rounded ends, usually occurring singly or in pairs, less frequently in long or short chains. The latter are found in the pseudo-zoogloea formed on the surface of beef-broth and other liquid media.

No spores or capsules were observed. The limits of size were found to be 1·5 to 5 μ by ·8 to 1·2 μ, the majority being from 2·5 to 3·5 μ in length.

The organism is actively mobile in a hanging drop culture made from an agar streak 1 to 4 days old; the rods occur singly, in pairs or in short chains, and the motion consists of darting or tumbling movements. The single rods do not progress far in one direction but make short, swift darts interrupted by tumbling movements. The chains move forward in a sinuous manner.

Examination with the dark ground illumination shows that the flagella are polar and that they are at the forward end of the rod as it moves.

These flagella stain readily by Ellis's modification of Loeffler's method. In the first preparation the majority of the rods showed two, three and four flagella, and, since Smith (8) describes the organism as possessing one polar flagellum and sometimes two, a large number of slides were prepared from different cultures. In these over 50 % of the rods had four flagella, a large number two or three, and a small minority had one only (Plate XXIV, fig. A). From the cultural characters it seems certain that the organism is the one described as *Bacterium mori* and this is the only morphological difference observed. Possibly when differently treated some of the flagella are dropped, but as Smith does not mention by which method his flagella were stained I was unable to ascertain whether this was the explanation.

The rods stain readily with carbol fuchsin and other aniline dyes but are Gram-negative. Involution forms were observed in broth containing 6 % NaCl.

Cultural Characters, etc.

Colonies on (+ 15) *nutrient agar* are barely visible to the naked eye after 24 hours at 25° C. After 48 hours they are round and white with a smooth margin; the margin subsequently becomes undulate. Surface colonies attain to a diameter of over 1 cm. in thinly sown plates, but the size is much affected by crowding. The internal structure of the colonies is at first homogeneous and later firmly granular. Submerged colonies remain very small and irregular in outline.

Nutrient agar streak. On slant agar (+ 15) there is a fair amount of growth which is smooth, white, flat and spreading with an entire margin. It is translucent, slimy and odourless; the medium is not stained.

Nutrient agar stab. A very thin white line of growth follows the needle track; the best growth is at the top.

Nutrient gelatine colonies (+ 15) are flat, white, slow-growing, more or less round; the margin is at first smooth then undulate-lobulate; no liquefaction.

Nutrient gelatine stab. There is no liquefaction; the best growth is at the top of the gelatine; on the depth of the medium there is no growth, even along the needle track. The medium is not stained and there is no odour.

Potato. On this medium the growth is thin, spreading, glistening, white to dirty white. The medium is slightly greyed; there is hardly any action on the starch.

Nutrient broth (+ 15) rapidly becomes turbid; a pellicle is formed which breaks up and falls to the bottom of the tube, forming a thick, white, flocculent sediment.

Litmus milk becomes blue rather rapidly though not so quickly as with *B. campestre.* There was no coagulation or other change during the two months the tubes were under observation. The reaction is continuously alkaline.

Cohn's solution. No growth.

Fermentation tubes. There were no gas formation and no clouding of the closed arm in beef broth containing 2 % of the following carbohydrates: dextrose, saccharose, lactose, maltose, glycerine and mannite.

Indol. No reaction for indol except a doubtful one in a single tube.

Nitrates. There was no reduction of nitrates in nutrient broth containing potassium nitrate.

Sodium chloride. The organism grew vigorously in nutrient broth containing up to 6·5 % sodium chloride; feebly in broth containing 6·5 % to 7·5 %. Tubes containing higher percentages remained clear.

Chloroform. The organism can grow vigorously and for a long time in broth over chloroform; but in a number of cases when not copiously inoculated it failed to do so.

Temperature. The organism grows well at 25° C. and also at 20° C. It was killed by 10 minutes' exposure in thin glass tubes to a temperature of 50° C. Smith gives a slightly higher death point, 51½° C. Tubes exposed to a temperature of 48° C. rapidly became turbid.

Desiccation. Cultures were obtained from leaves which had been drying in the air of the laboratory for 12 months. The resistance of the organism to drying on cover glasses was not tested.

Sunlight. Thinly sown plates were exposed to bright sunlight bottom upwards on ice. The rods were all killed in those exposed for 30 minutes; there was considerable reduction in the number of colonies in those exposed for 15 minutes, and still more after 25 minutes. The exact percentage was not estimated.

SUMMARY.

1. The black mulberry (*Morus nigra*) is very subject in South Africa to a blight affecting twigs and leaves.

2. The blight is fairly widespread but certain districts, particularly the western part of the Cape Province, are as yet free from it.

3. Spraying with Bordeaux mixture is useless in controlling the disease.

4. The organism causing the blight was isolated and numerous infections obtained with pure cultures.

5. The morphological and cultural characters of the organism correspond with those of *Bacterium mori* which causes the French and American mulberry blight.

6. The bacterium as isolated from leaves of blighted trees in South Africa has one to four polar flagella. This is the only important variation from the organism as described in America. Smith describes it with one, sometimes two, polar flagella.

BOTANICAL LABORATORIES OF THE UNION OF SOUTH AFRICA,
PRETORIA.

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The investigation which forms the subject of this paper was carried out in the Union Phytopathological Laboratories which are under the direction of Mr I. B. Pole Evans, M.A., B.Sc., F.L.S., Chief of the Division of Botany.

I have to thank Mr R. A. Davis, Chief of the Division of Horticulture, for naming the varieties of *Morus* and for valuable information as to the distribution of the disease.

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EXPLANATION OF PLATES.

PLATE XIX.

Fig. A. Photograph of mulberry leaf two months after inoculation with *Bacterium mori*. The diseased tissues have fallen away, leaving the leaf in a ragged condition.

Fig. B. Leaf five days after inoculation, showing a number of small spots in the water-soaked stage.

PLATE XX.

Fig. A. Leaves two months after inoculation, showing distortion due to the disease.

Fig. B. Twigs of *Morus nigra*; the terminal parts of the branches have been killed by the blight.

PLATE XXI.

Figs. A and B. Two photographs of a twig of the common mulberry, showing a number of dark, sunken spots due to infection with *B. mori*. The larger infection at the base of the twig was the result of a needle prick.

PLATE XXII.

Section through water-soaked spot on leaf of *Morus nigra*. Drawn with Edinger's projection apparatus.

PLATE XXIII.

Section through water-soaked spot on a leaf of the common mulberry, fixed five days after inoculation. Drawn with Edinger's projection apparatus. In Plates XXII and XXIII and XXIV B the dots or small strokes are only intended to represent the position of the bacteria, and in no way indicate the comparative size of the rods.

PLATE XXIV.

Fig. A. Rods from a 24-hour old culture on nutrient agar, treated with Ellis's modification of Loeffler's flagella stain. Drawn with the aid of the camera lucida, a Zeiss 1/12 imm. objective and compensating ocular No. 12.

Fig. B. Bacteria in the intercellular spaces; detail from Plate XXIII.

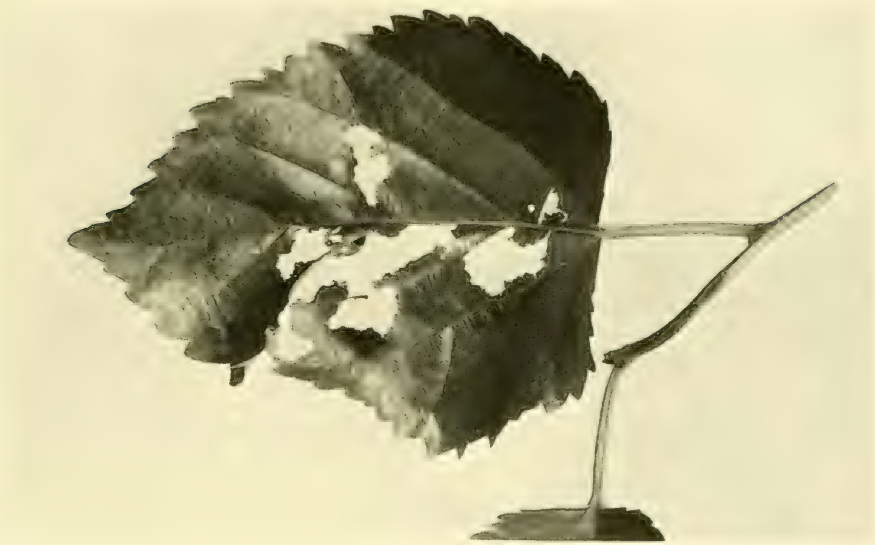


Fig. A

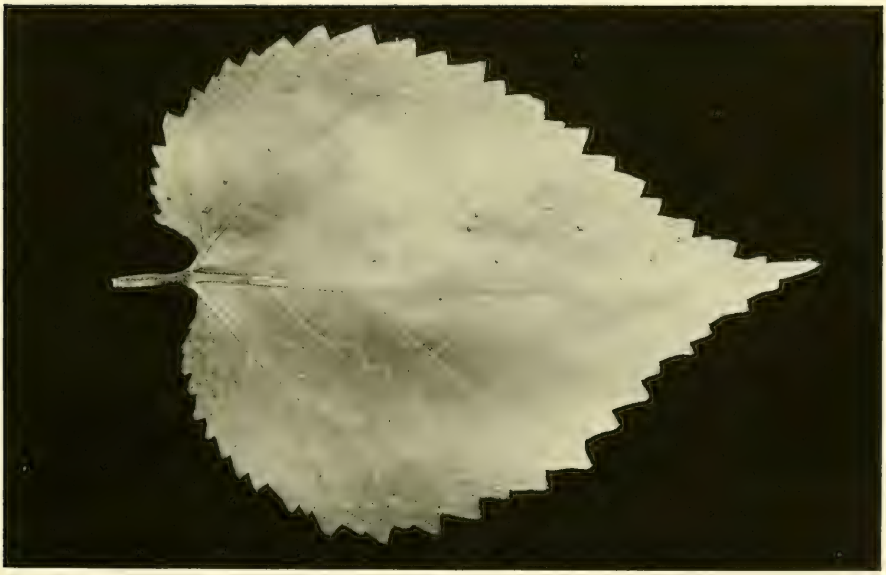


Fig. B

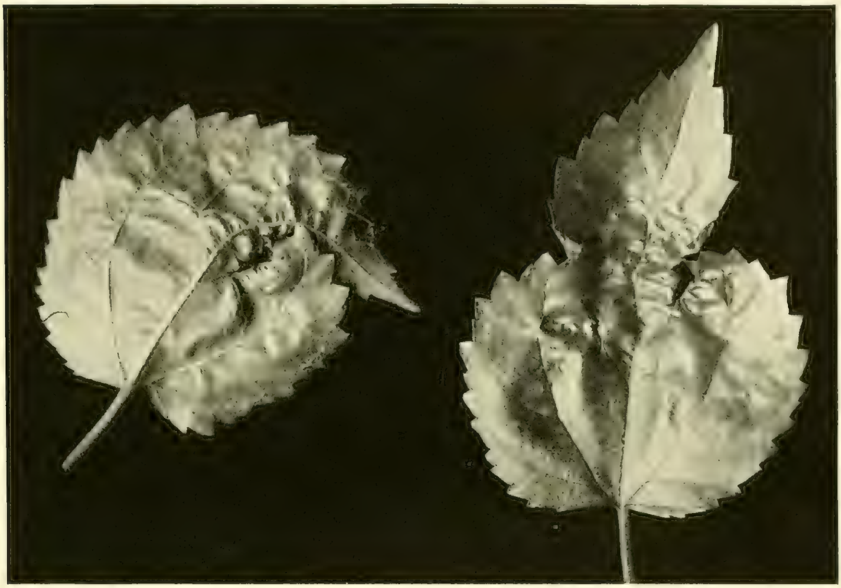


Fig. A

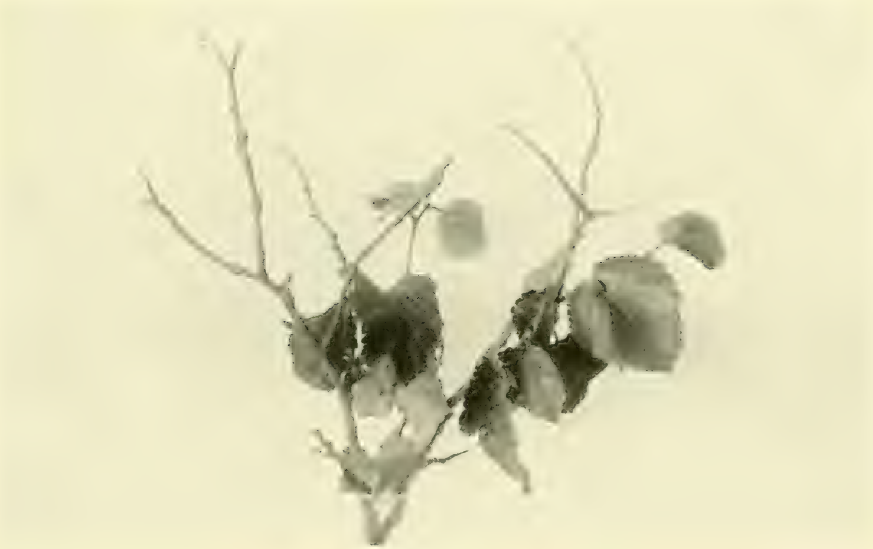


Fig B

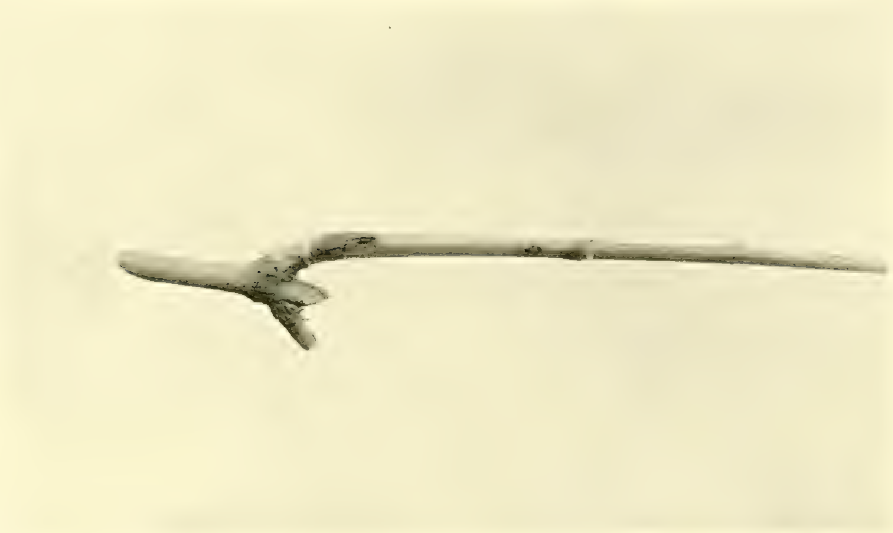


Fig. A

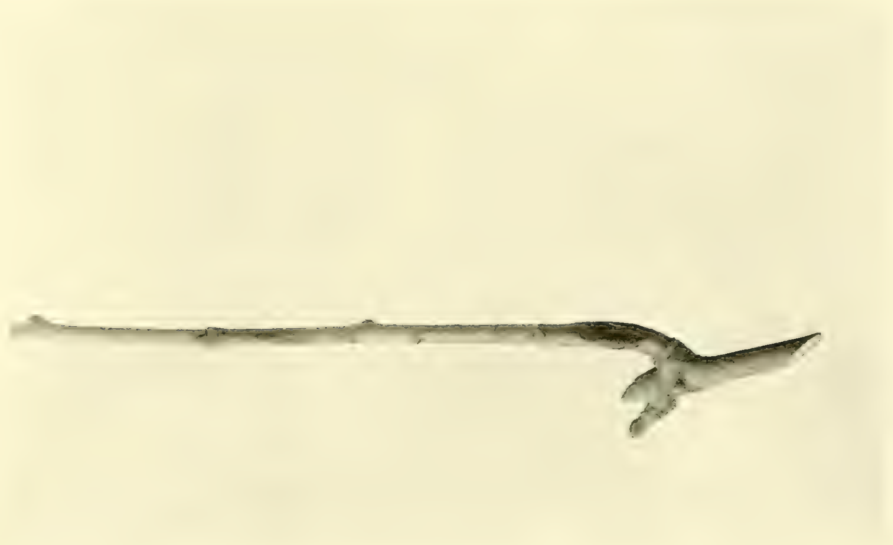
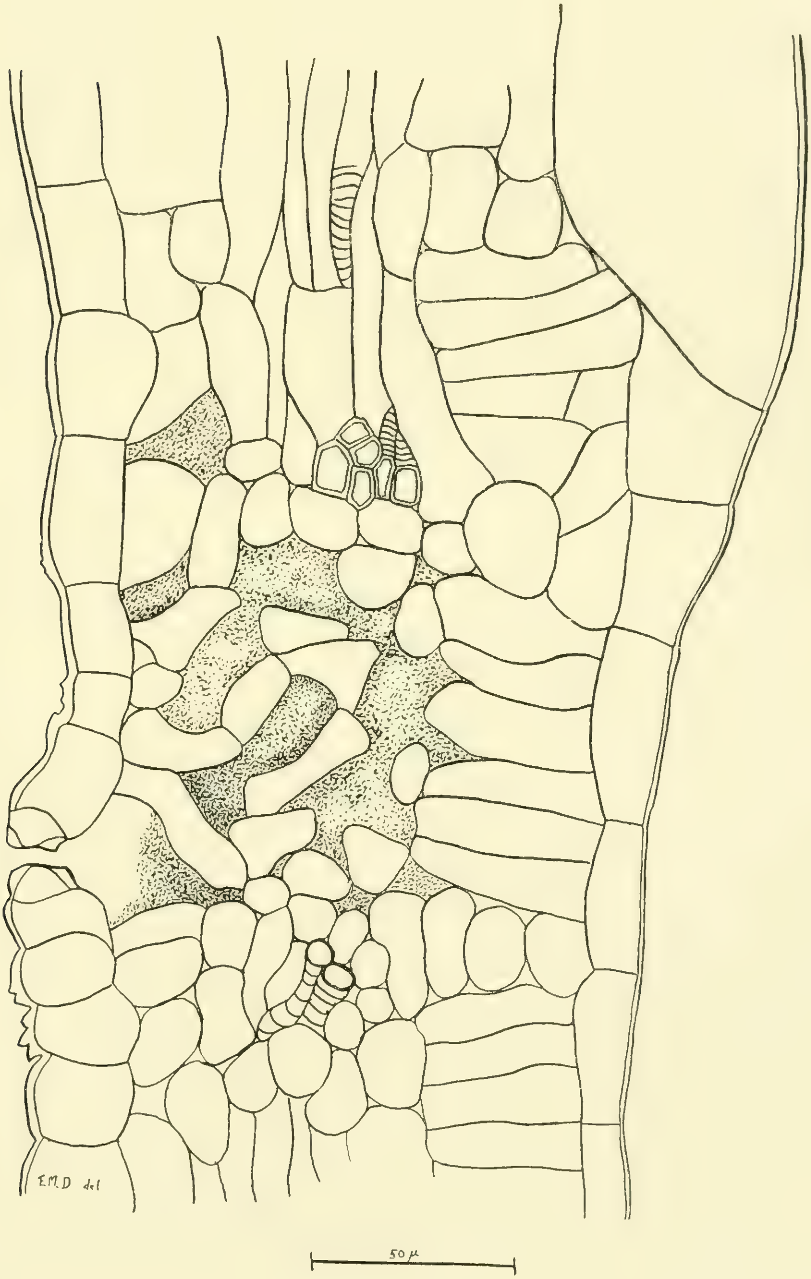
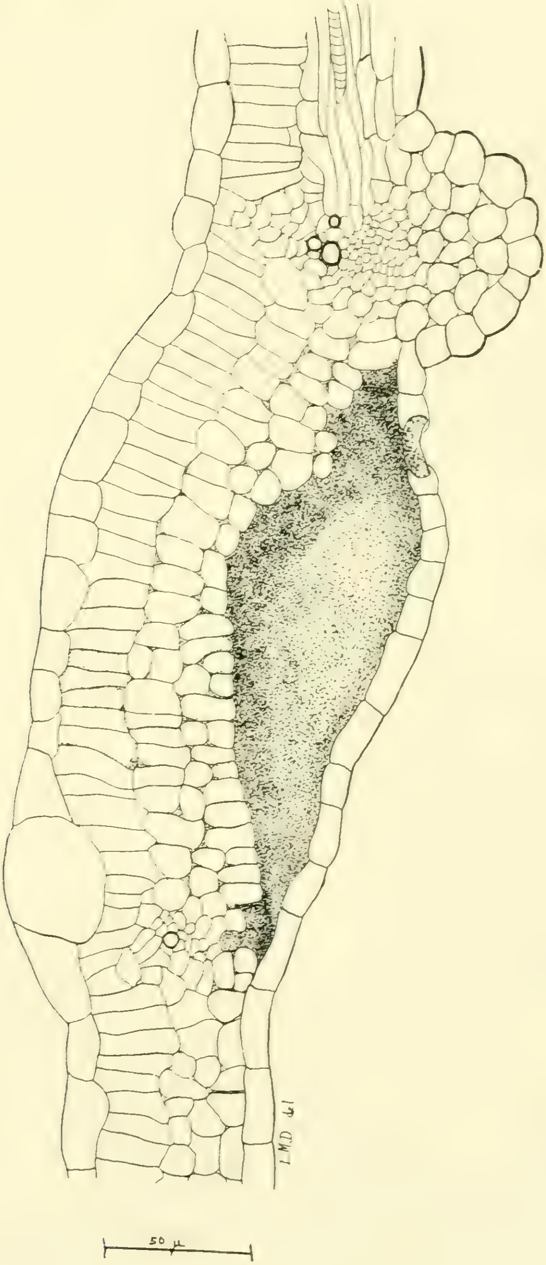


Fig. B


E.M.D del
50 μ


LMD del
50 μ

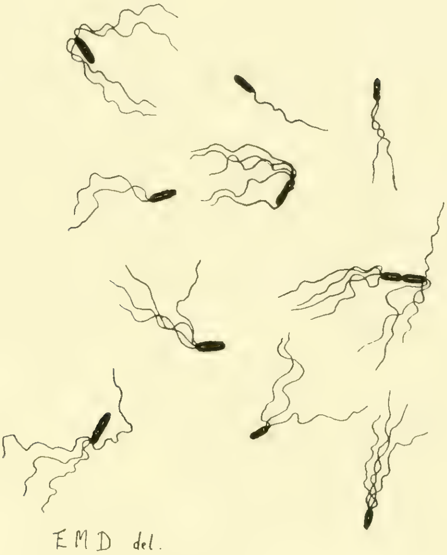


Fig. A

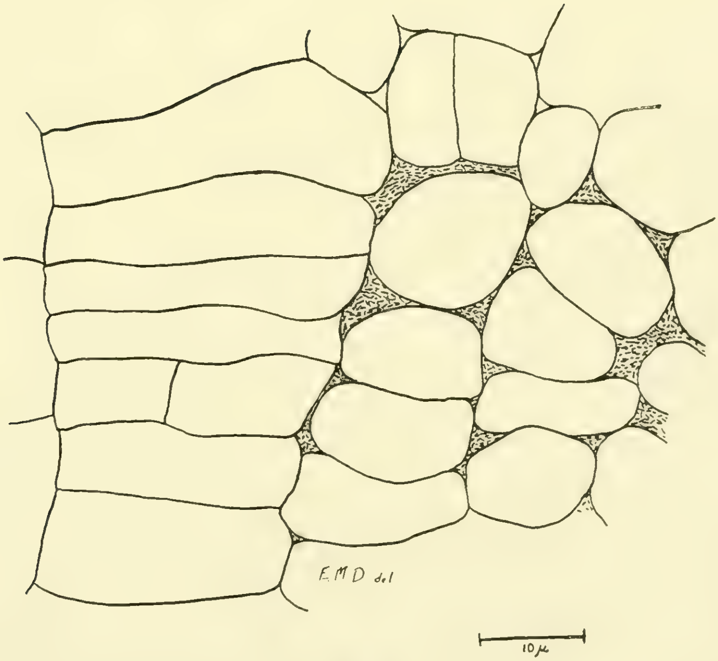


Fig. B

"BLACK NECK" OR WILT DISEASE OF ASTERS.

By WILFRID ROBINSON, M.Sc.

Lecturer in Economic Botany, Manchester University.

(With Plates XXV, XXVI.)

The wilt disease of China Asters is extremely prevalent in market gardens around Manchester, being locally known as "Black Neck" or "Black Leg" disease, and in recent years it has attacked and destroyed large quantities of asters in the district. My attention was drawn to the disease by a large grower of asters at Northenden late in the season of 1913, but the plants then examined were so badly affected that it was impossible to determine accurately the causal organism. Several fungi were isolated from the dead plants but owing to lack of suitable material for inoculation experiments the investigation did not at that time proceed further. Early in 1914, however, the same grower notified me that his seedling asters were seriously diseased, and in the course of a very short time some thousands were destroyed. The seedlings thus attacked formed the starting point for the study of the disease which was under observation in this garden throughout the season.

It soon became clear that the aster may show the characteristic symptoms of the disease at any period in its growth. While many seedlings succumb completely without growing further, the infected plants often continue their growth and may even reach the flowering stage before they collapse. The leaves of such older plants show clear signs of flagging from below upwards, while seedlings exhibiting the same symptoms often droop and damp off. The lower part of the stem shows a very distinct browning or blackening of the tissues for a short distance above the ground level and in many cases the whole of the cortical tissues are decaying. These decaying tissues form an extremely suitable medium for the growth of the various saprophytic fungi, which are usually found in abundance in the latest stages of the disease. The roots of infected plants are shrivelled and decayed, but infected seedlings often produce new roots so postponing the effects of the attack.

This disease is prevalent wherever asters are grown, but a complete study of it does not appear to have been made. It was first described by Galloway[1] in America in 1896, an undetermined species of *Fusarium* found upon the roots being named as the causal organism; W. G. Smith[2], in this country, at a later date attributed a disease having similar symptoms to a fungus possessing oval spores, which, however, were not figured nor was the species identified. R. E. Smith[3] again in America described the disease, associating it with a fungus which blocks the conducting tissues of the vascular bundles, but as before the fungus was undetermined. More recently Osterwalder[4] has given *Fusarium incarnatum* as the cause of the disease, but having failed to see his paper or an abstract of it I have no knowledge of the evidence upon which his conclusions are based. Massee[5] has described a disease of sweet peas, asters, and other plants which he attributed to *Thielavia basicola*. He found that asters were always killed outright in the seedling stage but no inoculation experiments upon seedlings were described. In the present investigation *Thielavia basicola* has never been observed upon diseased seedlings or older plants.

On the other hand, Friend[6] in 1897 ascribed it to the attacks on the roots by an organism which he named the Aster Worm (*Enchytraeus parvulus*). W. G. Smith[7] also found nematode worms living on the decaying parts of diseased plants. Occasionally I also have observed these worms on diseased roots, but the infection experiments described below indicate that this is not the direct cause of the wilt disease.

Among the saprophytic fungi present on the rotting roots and the lower parts of the stem of badly diseased plants there is usually found a species of *Fusarium*, and this fact seems to have led to its identification as the causal organism of the wilt disease. Up to the present, however, no account of any infection experiments with this fungus on living asters has been given. The results of such experiments described in this paper indicate that while the *Fusarium* may be a secondary or accessory factor, it is not the primary cause of the disease. It has already been mentioned that infected seedlings show "damping off," and this is invariably due to a species of *Phytophthora*, the *Fusarium* not being present in the tissues in the early stages of the disease. This *Phytophthora* always occurs in the tissues of infected asters even when the latter are mature and the characteristic mycelium is easily recognised in sections made near the upper limit of the diseased part of the stem. It will be convenient to describe first the method by which this *Phytophthora* was isolated and grown in pure culture, then to describe its main characters, and finally to give an account of inoculation experiments both with this fungus and the *Fusarium*.

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[1] *American Gardener*, vol. 17, p. 518.

[2] *Gardeners' Chronicle*, July 1900, p. 75.

[3] *Bull. Mass. Agric. Coll.*, 1902.

[4] *Landw. Jahrb. Schweiz.*, Bd. 24, 1910, pp. 247–8.

[5] *Kew Bulletin*, 1912.

[6] *Gardeners' Chronicle*, 1897 (August).

[7] *loc. cit.*

The *Phytophthora* was first detected by the following experiment. A diseased seedling was cut off through the hypocotyl so that a small portion of the infected region was included in the separated part. This seedling was then placed with the cut end in water and examined at the end of 24 hours. In this time an abundant crop of characteristic pear-shaped sporangia had developed from near the cut surface. The similarity of these bodies to the sporangia of certain species of *Pythium* and *Phytophthora* led to the suspicion that one of these Phycomycetes was probably the cause of the disease in asters. It will be seen that all the later work, involving a careful comparison of the morphological characters of the mycelia of the *Phytophthora* and the *Fusarium* as well as experimental infections with both fungi, confirmed the correctness of the above suspicion.

Methods. Observations were carried out on living material from diseased asters of different age, on the fungus grown in pure culture, and also upon carefully fixed material. The last named was prepared by fixing very small pieces of diseased tissue in Flemming's weaker solution, or in chromo-acetic acid weak solution diluted with water to one half strength. These pieces were embedded, cut in serial section, and stained either with Flemming's triple combination or Heidenhain's Iron-alum Haematoxylin followed by Orange G or Bismark Brown. Delafield's Haematoxylin was also found useful for bringing out the cellulose walls of the hyphae in the tissues. Sections from living material were fixed in Iodine and examined in Schultze's Chlor-zinc-iodide solution.

Pure cultures were made on several different media, viz. Aster agar, Beerwort agar, Quaker Oat agar, French Bean agar, Tomato agar, and Salep agar. The aster agar was made by cutting up four healthy, almost full-grown aster plants, and boiling for half an hour in 500 c.c. water. The mixture was then filtered, 10 grams of strip agar added, and the medium sterilised in the autoclave. The other media were prepared by the ordinary methods given in recent papers by Pethybridge[1], Dastur[2] and others. A more or less healthy growth of the fungus was obtained on all these media but that on Quaker Oat agar was by far the most vigorous. Here an aerial mycelium soon formed a dense woolly felt over the surface of the medium, whilst on other media the growth was much less rapid, aerial hyphae being only sparingly produced.

The fungus was isolated in the following way. A diseased plant was well washed, first in water and then rapidly in a saturated solution of corrosive sublimate. The portion of the stem near the upper limit of infection, as indicated by the discoloration of the tissues, was cut off with a razor previously sterilised. Sections were cut longitudinally from this piece of stem and transferred to the surfaces of Aster agar and Beerwort agar in Petri dishes. After 48 hours the mycelium had spread a considerable distance over the surface of the medium and the growth near its limits was free from bacteria and other fungi. From this region portions of the mycelium were transferred to tubes and plates of sterile media and the cultures in these remained pure. Owing to the very rapid growth on Quaker Oat agar it was necessary on this medium to start fresh sub-cultures fortnightly. New cultures were also started from time to time, by the method described above, from different diseased asters, and in every case the fungus isolated was the *Phytophthora* already referred to.

Although over a hundred artificial cultures have been made up to the present, no mature sporangia have appeared on solid media. The sporangia, however, were obtained in abundance by transferring portions of vigorously growing mycelium to the roots of various seedlings submerged in water in Petri dishes. Among seedlings used successfully for this purpose were those of *Aster*, *Helianthus*, *Gilia*, *Lycopersicum esculentum* and *Senecio vulgaris*. After about three days very abundant crops of sporangia were formed on these pieces of mycelium. The liberation of zoospores was then easily observed by removing the hyphae bearing mature sporangia to hanging drops of fresh well-aerated tap water. It is possible also to obtain sporangia by simply bringing pieces of mycelium from pure cultures into tap water, but under these conditions they do not appear for 16 to 21 days.

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[1] *Sci. Proc. Roy. Dublin Soc.*, 1913–14.

[2] *Mem. Dept. Agric.*, India, 1913.

The Mycelium in the Tissues.

The initial infection of aster plants by the *Phytophthora* occurs in the seedling stage through the roots and root-hairs. This was proved by observations on seedlings shortly after infection as well as by the infection experiments which are described below. Many diseased seedlings wilt and succumb almost immediately but others, though infected by the fungus, continue to grow and may even reach the flowering stage before wilting. A comparison of sections of the stem of diseased asters of various ages shows that the wilting, both of seedlings and of older plants, results from the extension of the mycelium to the tissues of the vascular cylinder. The presence of the fungus in the older plants, even though the wilting is delayed, invariably produces serious dwarfing. This effect is obvious in Plate XXV, which is a drawing[1] to scale from a photograph of two plants of the same age, the one attacked by the fungus and the other free from it. Both plants were grown side by side under similar conditions in the same bed.

Transverse and longitudinal sections of diseased plants of different ages show that the mycelium advances upwards in the cortex and for a time at least the cells of these tissues remain living and turgid. The mycelium grows both in the intercellular spaces and through the cells, and in the latter case, when entering or leaving a host cell, a hypha shows a distinct constriction where it passes through the cell wall (Plate XXVI, figs. 1 and 2). Suitably stained preparations show that the hyphae penetrate through the small pits which are frequent in the cell walls (Fig. 3). Haustoria rarely occur, if indeed they are present at all. This is difficult to decide with certainty since branches of the hyphae grow through the cells and the apparent haustoria may simply represent such young branches. Sooner or later the mycelium extends to the vascular bundles. The thin-walled tissues of the phloem especially are attacked and rapidly killed; the hyphae penetrate into the cells of the medullary rays (Fig. 4) and even occasionally send branches through the vessels (Fig. 5). The passages of the last named, however, are not directly clogged by the fungus as was suggested by R. E. Smith[2]; but the proximity of abundant mycelium to the vessels, as well as the drain on the phloem and medullary rays, is sufficient to account for the wilting.

The mycelium consists of sparingly branched hyphae from 3μ to

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[1] I am indebted for this drawing to my brother Mr J. B. Robinson.

[2] *Bull. Mass. Agric. Coll.*, 1902.

9μ in diameter, and when young these are non-septate. In later stages and especially in pure cultures septa appear irregularly. The hyphae are multinucleate and have the nuclei disposed at fairly regular intervals (Fig. 2). The structure of the nucleus is somewhat difficult to make out even with the best nuclear stains, but it clearly contains a single nucleolus (Fig. 6). In addition to the nuclei, the vacuolated cytoplasm contains numerous regularly arranged granules which stain readily with nuclear stains (Figs. 6 and 8). Tests with osmic acid and Scharlach R indicated that these granules are not of the nature of fatty bodies. The second of the tests showed fat in the vacuoles of the cytoplasm; the deeply staining granules may therefore correspond to the chondriosomes described and figured by Lewitsky[1] in the young oogonia of *Cystopus Bliti*. Further investigation will be necessary to show whether such an interpretation is correct, but in this connection it is of interest that the granules in question persist in the sporangia and zoospores (Fig. 19).

The walls of the hyphae and also of the sporangia are of pure cellulose and are readily stained violet by Schultze's chlor-zinc-iodide solution. This reaction afforded a ready means of recognising the fungus throughout the investigation, especially when questions of comparison with the *Fusarium* were being considered. The mycelium of the latter stains a deep yellow with this reagent and is therefore readily distinguishable from the *Phytophthora*.

Sporangia.

The sporangia of this fungus have never been observed to be formed except under water; in this respect the species resembles *Phytophthora omnivora* (De Bary[2]) and *Ph. erythroseptica*[3] recently described by Pethybridge as causing the pink rot of potatoes. Prior to the formation of sporangia the vegetative hyphae in the host tissues turn outwards and grow through and between the cells until they reach the epidermis (Fig. 7). They pass through the epidermal cells but the cuticle (*c*) apparently offers some temporary resistance to further progress for each hypha then swells out into one or more rounded branches (Figs. 7 and 8). These gradually separate the cuticle from the epidermis and often form characteristic sorus-like groups (Fig. 7). Each of the swollen bodies is densely filled with protoplasm, contains several nuclei and also large numbers of the deeply staining granules already referred to (Fig. 8). Up to this point, however, no septum has appeared below the swollen portion. The apex of each body becomes very closely pressed against the cuticle and then penetrates the latter by a fine hypha which at once grows out to produce sporangia. The outgrowing hypha may branch at the point of exit (Fig. 9) and produce several sporangiophores or merely give rise to one. Frequently only a single terminal sporangium is borne upon such a sporangiophore, but in many instances series of three or four sporangia arise successively in a sympodial manner. This mode of production of the sporangia is characteristic of several species of *Phytophthora*.

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[1] *Berichte der Deutsch. Bot. Ges.* Bd. 31. H. 9, 1913.

[2] *Bot. Zeitung*, 1882.

[3] Rotting of potatoes by a new species of *Phytophthora*, *Sci. Proc. Roy. Dublin Soc.*, 1913. Further observations on *Phytophthora erythroseptica*, *Sci. Proc. Roy. Dublin Soc.*, 1914.

The sporangium arises as a slight swelling of the tip of the hypha bearing it, and this swelling gradually enlarges, becoming first globular and then oval in shape (Fig. 10 *a*, *b*, *c*). At maturity it measures on an average 32μ by 60μ, being then separated from the hypha bearing it by a transverse septum. The apex of the sporangium does not always show a very definite papilla as in other species of *Phytophthora*. As it approaches maturity a large central vacuole appears (Fig. 12) and this corresponds in position and appearance to the central oil body described by Pethybridge[1] in *Ph. erythroseptica*. A little before maturity the vacuole disappears and then the contents of the sporangium are seen to have divided up into 13 to 15 zoospores (Figs. 13, 14, 15). The sporangia germinate while still attached to the stalk and have never been observed to fall off as in *Ph. infestans*. Germination of the sporangia may take place either by the liberation of motile zoospores (Fig. 18) or under different conditions by the direct production of a germ tube (Fig. 17). This corresponds to the observations of De Bary[2] and of more recent investigators on different species of *Phytophthora*.

Reference has already been made to the method by which the liberation of zoospores was observed. The discharge takes place immediately on transferring ripe sporangia into fresh well-aerated tap water. It is brought about by the solution or the opening of the apical portion of the sporangium, and a vesicular swelling-out of the apex has never been observed. The zoospores mature within the sporangium and are directly discharged in a mass, in groups of two or three or one after another. As has been observed by many investigators in other species of *Phytophthora*, some of the zoospores occasionally fail to be discharged but germinate within the sporangium (Fig. 16). In some cases the discharged zoospores at first remain aggregated together and show very sluggish amoeboid movements, altering their shape as they move. Gradually, however, they separate from one another and begin to swim about vigorously as most of the zoospores do from the first. In motion the zoospores continually alter their shape but generally are somewhat kidney-shaped with a median furrow (Fig. 18). They possess two vacuoles which appear to lie one on either side of the furrow, and two unequal cilia springing laterally from the middle of the depression. When moving the zoospore progresses in the direction of its longer axis, one cilium lashing forwards and the other trailing behind. After swimming for about half an hour they come to rest (Fig. 19) and immediately begin to germinate by means of a germ tube which soon branches (Fig. 20). In water alone these germ tubes do not grow much further, but on nutrient media they give rise to a normal mycelium. In hanging drops containing a piece of the root of an aster seedling, the germ tubes almost immediately penetrate the tissues and produce a mycelium within.

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[1] *loc. cit.*

[2] *loc. cit.*

After the liberation of the zoospores the hypha forming the stalk of the sporangium grows into the empty sporangium and then forms a new sporangium within the first (Fig. 21). As many as three sporangia are in some instances thus formed within one another (Fig. 22). Variations also occur in which the proliferating hypha grows out of the empty sporangium before forming the new sporangium (Fig. 22). So far as I am aware this proliferation of sporangia has not been previously described for any species of *Phytophthora*, although De Bary[1] describes and figures similar examples in *Pythium proliferum* and *P. megalacanthum*.

Fusarium.

The vegetative characters of the species of *Fusarium* so frequently present on the decaying portions of the stems of diseased asters render this fungus easily distinguishable from the primary cause of the disease. The mycelium branches much more freely than that of the *Phytophthora* (Figs. 23 and 24), it stains yellow with iodine and also with Schultze's solution and its walls give none of the reactions characteristic of cellulose. The hyphae are abundantly septated, with individual cells containing several nuclei and characteristic oil globules, but none of the regularly disposed granules that are invariably seen in the *Phytophthora*. Conidia are produced in great abundance, are typically four- or five-celled, curved and slightly pointed at the ends (Fig. 24). Although carefully looked for the mycelium of this fungus has never been observed among the living cells of the host plant. On the other hand it is always possible to find the mycelium of the *Phytophthora* in the higher parts of the diseased regions, that of the *Fusarium* always being confined to the lower decaying parts of the stem and to the roots. This *Fusarium* was isolated and grown in pure culture on Beerwort and Quaker Oat agars.

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[1] *Bot. Zeitung*, 1881, pp. 559–623.

Experimental Infections.

A number of infection experiments was carried out on seedling asters. As a preliminary test, from a number of seedlings growing together in a pot one was selected and a small quantity of mycelium of the *Phytophthora* from a pure culture of the fungus was placed near the collar. After five days the seedling had completely collapsed showing the ordinary symptoms of "damping off." All the other seedlings in the pot remained unaffected for at least 10 days after the experiment. On cutting off the collapsed seedling and placing it in water, hyphae grew out of the tissues and produced typical sporangia in 24 hours.

For more critical experiments a number of aster seedlings were carefully uprooted, washed free from soil and laid in Petri dishes with the roots in water. Three of these were then inoculated by placing on each of the roots a piece of mycelium from a pure culture on Quaker Oat agar. Controls were placed in Petri dishes with the roots in water without being inoculated. Zoosporangia were abundantly produced on the mycelium and the seedlings became infected, the hyphae having travelled one inch in the tissues of the hypocotyl at the end of nine days. The tissues were browned and the seedlings were beginning to collapse. The controls appeared quite normal at the end of the same period and no fungus was present in the tissues.

An exactly parallel series of tests with controls was carried out on seedlings of asters, spores from a pure culture of *Fusarium* (previously isolated from decaying roots of diseased asters) being used for inoculation. After nine days no change was observable and up to the 19th day, when the tests were stopped owing to the complete collapse of the seedlings in the *Phytophthora* inoculations, the *Fusarium* series remained healthy and normal.

Further series of tests were carried out by inoculating seedlings grown on moist cotton wool in test tubes, with mycelium of the *Phytophthora* and spores of the *Fusarium* as before. Here also the seedlings inoculated with *Phytophthora* collapsed after 9 to 12 days, while those inoculated with the *Fusarium* and also the controls remained unaffected in any way. The Petri dish inoculations were repeated several times and it was invariably found that the seedlings inoculated with the *Phytophthora* showed the characteristic symptoms of the disease under investigation. Sections of such seedlings always showed that the collapse was due to the presence of the mycelium of that fungus in the tissues. The *Fusarium* on the other hand never produced such effects, in fact it was only possible to grow this fungus on decaying seedlings or on those previously wounded.

The stems of almost mature aster plants were also inoculated with mycelium of the *Phytophthora*, a slight wound being made with a sterile scalpel and a piece of mycelium from a pure culture inserted. Controls were also wounded but not inoculated. After 10 days the mycelium had progressed over one inch upwards in the tissues of the stem and had produced the dark discoloration characteristic of the disease. On cutting off one of these stems through the discoloured region, well above the inoculation wound, and placing it in water, the typical sporangia were obtained in 24 hours. It was not found possible to infect older asters without previously wounding the tissues.

Conclusion.

No sexual organs of any kind have yet been found either on the fungus grown in pure culture or upon asters at any stage of the disease. All attempts to produce the oogonia or antheridia in pure cultures by the methods successfully used by Clinton[1], Pethybridge[2], Dastur[3], Klebahn[4] and others for various species of *Phytophthora* have so far proved unsuccessful. The characters already described appear sufficiently striking, however, to warrant a discussion of the systematic position of the fungus to which I attribute the disease of asters described above. Reference has already been made to the close resemblance of the vegetative mycelium and sporangia to those of *Pythium* and *Phytophthora*. A close examination of the characters of these two genera reveals the fact that the main points of difference lie in the modes of formation and liberation of the zoospores. In *Pythium* the sporangium swells out to form a large apical vesicle into which the undifferentiated contents of the sporangium pass. The zoospores then become defined and are liberated by the rupture of the vesicle. In *Phytophthora*, however, no vesicle is formed and the zoospores round off within the sporangium some time before their discharge which takes place directly by the solution or rupture of the apex. In these details therefore the fungus under consideration corresponds to *Phytophthora* and the very large size of the sporangium approximates it to *Phytophthora omnivora* (De Bary). *Ph. omnivora* was described by De Bary attacking a large variety of plants including *Clarkia*, *Gilia*, *Cleome*, *Schizanthus*, *Fagopyrum*, *Oenothera*, and *Epilobium*. A number of other investigators have more recently described fungi morphologically almost identical with *Ph. omnivora*, but owing to differences in the range of hosts which they will attack these have been separated off as distinct species. Examples of species founded mainly upon this physiological distinction are *Ph. cactorum* (Schenk), *Ph. Fagi* (Hartig), *Ph. omnivora* var. *arecae* (Coleman), *Ph. Faberi*, and *Ph. Syringae* (Klebahn). The characters of these various species and their affinities are so completely discussed in recent papers by Pethybridge[1], Dastur[2] and Butler[3] that a further recapitulation is unnecessary here. In this connection, however, it is of interest that I have already found it possible to infect and to obtain sporangia on seedlings of *Gilia tricolor*, *Ricinus*, and *Helianthus annuus* by inoculation with mycelium from pure cultures. Inoculations upon seedlings of *Lepidium*, *Lycopersicum esculentum* and young plants of *Solanum tuberosum* gave negative results in all cases. The failure to obtain oospores in cultures or even in infected seedlings of *Gilia* (in which De Bary found those of *Ph. omnivora* in abundance) indicates that the aster *Phytophthora* is not identical with *Ph. omnivora*, yet it seems likely that it is a physiological form of it. Further research will show whether the sexual organs are of the character described by De Bary for *Ph. omnivora* and whether the range of host plants is as wide as in the case of some of the above species. For the present, therefore, the question of the identity of the species of *Phytophthora* here described for the first time as producing the wilt disease of asters is deferred; but this investigation establishes the fact that the causal organism of the disease is a species of *Phytophthora* and not a *Fusarium* as has generally been supposed. It is also clear that, though the initial infection occurs in the seedling stage, the disease may not be seriously manifest until late in the life of the plant. In this respect, therefore, the aster disease differs from other diseases caused by species of *Phytophthora* where the destruction of the host plant is very rapidly accomplished.

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[1] *Rep. Conn. Agric. Exp. Sta.*, 1911.

[2] *loc. cit.*

[3] *Mem. Dep. Agric.*, India, 1913, 4.

[4] *Krankheiten des Flieders*, 1909.

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[1] *Sci. Proc. Roy. Dublin Society*, 1913, 1914. *loc. cit.*

[2] On *Phytophthora parasitica* nov. spec., *Mem. Dep. Agric.*, India, 1913.

[3] Studies in Peronsporaceae *Mem. Dep. Agric.*, India, May 1913.

In conclusion I should like to express my indebtedness to Professor W. H. Lang for his advice and helpful criticism during the course of this investigation, which has been carried out in the Cryptogamic Botany Research Laboratory of the University of Manchester.

SUMMARY.

1. The tissues of asters attacked by the wilt disease always contain the mycelium of a species of *Phytophthora*; this fungus was isolated and grown in pure culture on various media.

2. Several saprophytic fungi including a species of *Fusarium* were isolated from the decaying roots of badly diseased asters but none of these is the primary cause of the disease.

3. A series of inoculation experiments with adequate controls showed that the *Phytophthora* could produce a disease on seedling and mature asters identical in every respect with the "Black neck" or wilt disease.

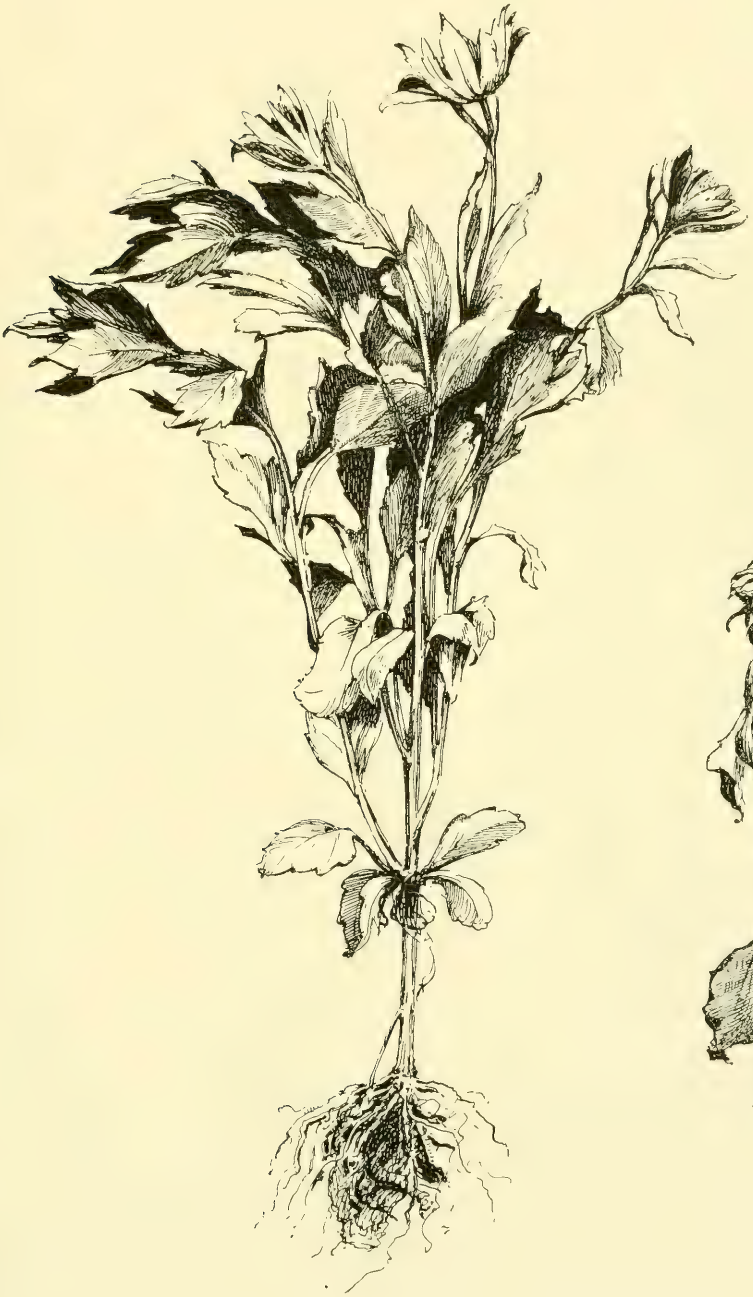
4. A similar series of inoculation experiments with the *Fusarium* gave negative results.

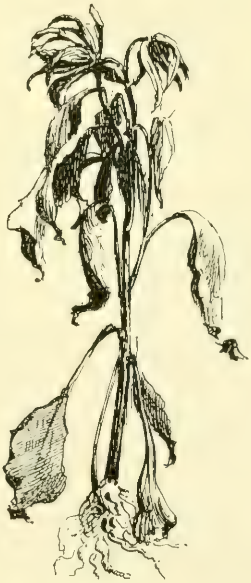
5. The characters of the vegetative mycelium and its relations to the tissues of the host plant were studied in some detail.

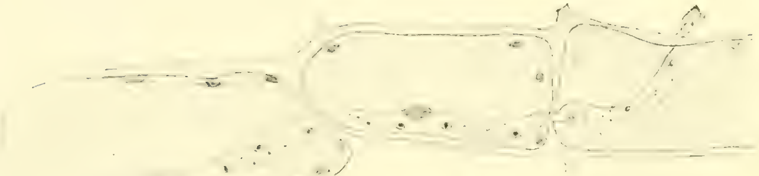
6. The sporangia show most of the characters described by De Bary for *Phytophthora omnivora*, but after the discharge of zoospores the stalk of the sporangium grows through and produces a second and even a third sporangium within the first. This proliferation has not, as far as I know, been previously described for any species of *Phytophthora*.

7. No sexual organs have as yet been observed either on infected plants or in pure culture on suitable media.

THE ANNALS OF APPLIED BIOLOGY. VOL. II, NOS. 2 and 3 PLATE XXV



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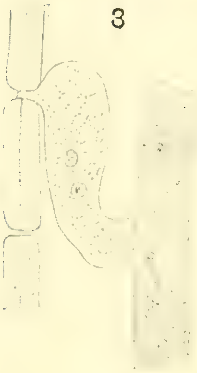
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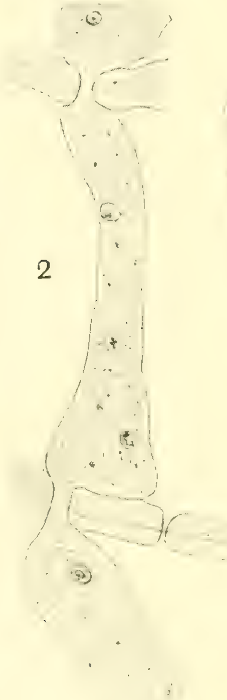
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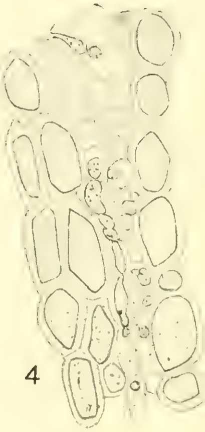
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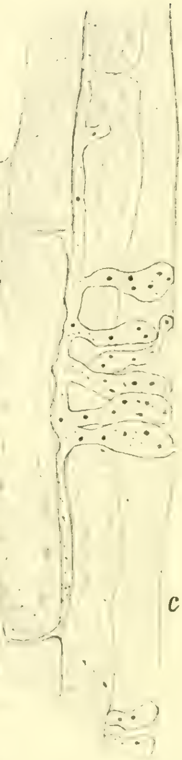
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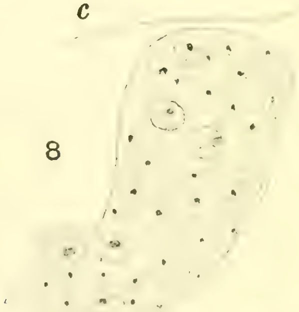
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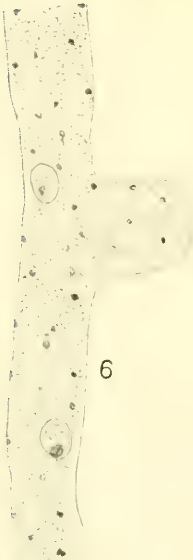
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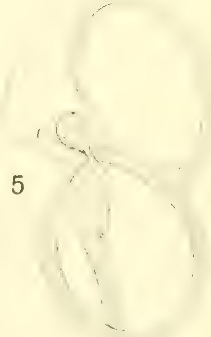
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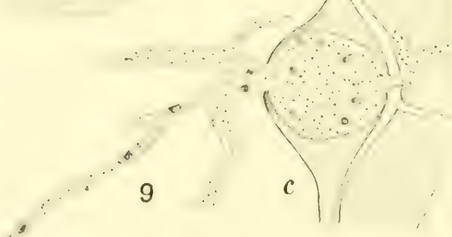
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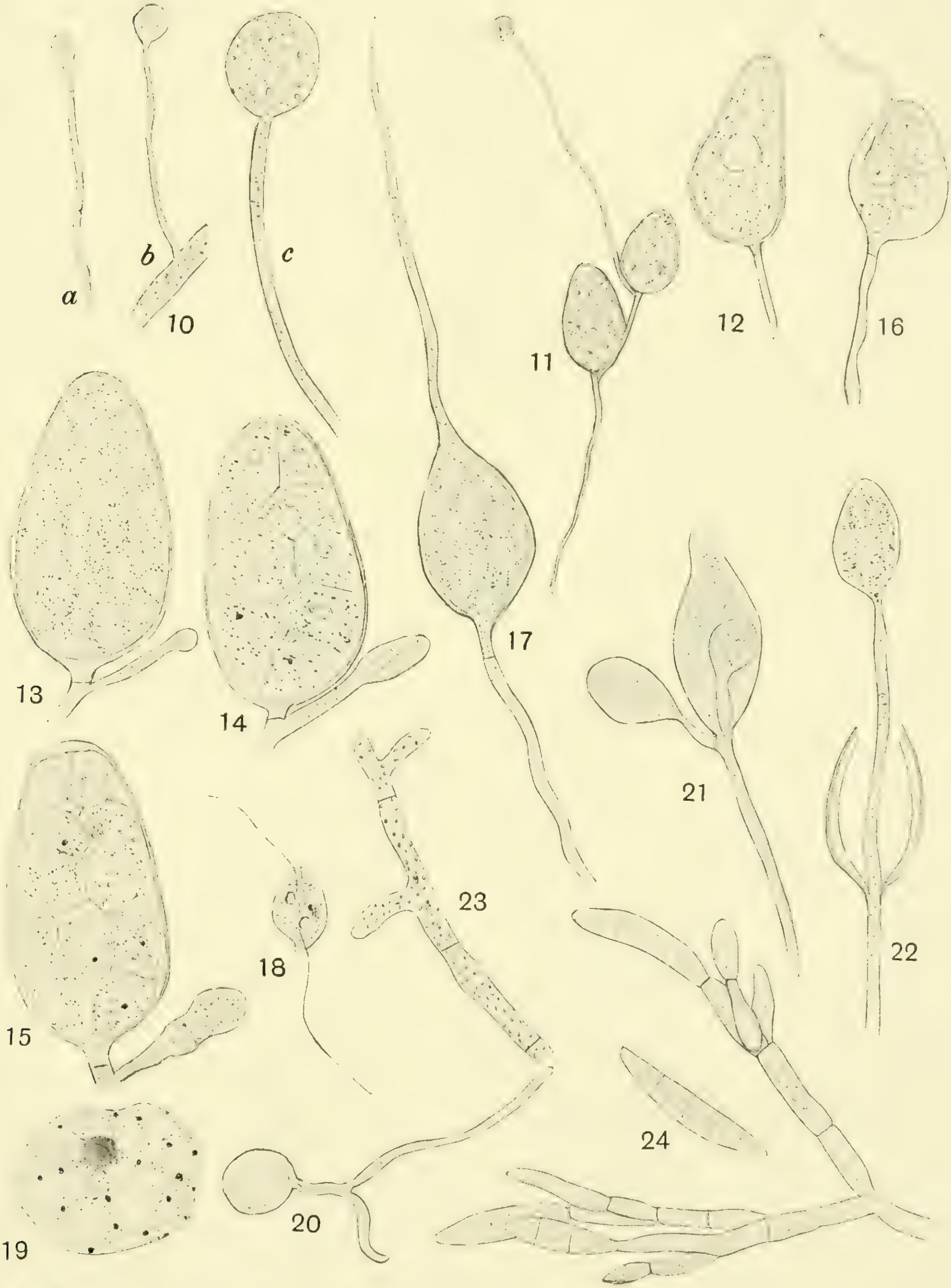
PLATE XXVI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DESCRIPTION OF PLATES.

PLATE XXV.

Two almost mature aster plants from the same bed—the one diseased and the other healthy—drawn from a photograph to scale.

PLATE XXVI.

Fig. 1. Cortical cells of the hypocotyl of seedling aster in *L.S.* showing hypha of *Phytophthora* growing through three cells. The hypha enters and grows through the protoplasm into the vacuole. × 180.

Fig. 2. Intracellular hypha passing through two parallel walls of a cell showing constrictions. × 1440.

Fig. 3. Similar hypha to that in Fig. 2 with tip of branch partially through a pit in the wall of a host cell. × 1440.

Fig. 4. Hyphae entering and growing through the parenchymatous cells of a medullary ray. × 180.

Fig. 5. Hypha entering one of the large vessels of the xylem seen in *T.S.* × 700.

Fig. 6. An intercellular hypha showing nuclei, vacuolated protoplasm and deeply staining granules at the junctions of the meshes of the cytoplasmic network. × 2000.

Fig. 7. *L.S.* of the outer portion of the stem of a diseased aster showing swollen hyphae separating the cuticle (*c*) from the epidermis, prior to the formation of sporangia on the exterior. One of the hyphae has penetrated the cuticle by a fine pore. × 180.

Fig. 8. One of the swollen hyphae similar to those in Fig. 7 showing several large nuclei and the deeply staining granules as in Fig. 6. *c.* = cuticle. × 2000.

Fig. 9. A later stage of a similar hypha to that in the previous figure showing branching as it leaves the cuticle. × 1440.

Fig. 10, *a*, *b* and *c*. Various stages in the formation of the sporangium. × 700.

Fig. 11. Sporangiophore bearing three sporangia in different stages showing sympodial development. × 570.

Fig. 12. Almost mature sporangium showing large central vacuole. × 570.

Fig. 13. Mature sporangium in which division into zoospores is beginning. × 700.

Fig. 14. The same sporangium as Fig. 13 ten minutes later. × 700.

Fig. 15. Same sporangium as in Figs. 13 and 14 drawn 15 minutes later. The zoospores have contracted somewhat from the wall and are more rounded. × 700.

Fig. 16. Sporangium in which some of the zoospores have failed to be discharged—one is seen germinating *in situ*. × 570.

Fig. 17. Sporangium germinating conidially by putting out a hypha. × 570.

Fig. 18. Zoospore as seen in motion showing unequal cilia and two vacuoles. × 700.

Fig. 19. Zoospore at rest stained to show nucleus cytoplasm and protoplasmic granules. × 2000.

Fig. 20. Germinating zoospore. × 700.

Fig. 21. Proliferating sporangium showing the stalk growing into the empty sporangium to form a second. × 570.

Fig. 22. Similar proliferating sporangium. There are seen the empty walls of two older sporangia which have discharged their zoospores. × 570.

Fig. 23. Portion of a hypha of *Fusarium*. × 700.

Fig. 24. Mycelium of *Fusarium* bearing conidia. × 700.

Figs. 10 to 18 and 20 to 24 on Plate XXVI were drawn from material in the living unstained condition.

A CONTRIBUTION TO OUR KNOWLEDGE OF SILVER-LEAF DISEASE.

By Ph. Dr JAR. SMOLÁK (Roudnici, Bohemia).

(*From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.*)

THE etiology of Silver-leaf disease has passed through many phases and has been the subject of many diverse opinions. First observed by Prillieux (1885), this disease was placed by Sorauer (17, p. 285, "Milchglanz") among the non-parasitic plant diseases. Similarly Delacroix (2, p. 227) places "le plomb" among the "maladies non-parasitaires." Percival (12), Güssow (3) and Brooks (1) record successful inoculation experiments with the fungus (*Stereum purpureum*). Massee (9) rejects this view of the etiology by *Stereum*; Blackmore ascribes the disease to the action of bacteria. Though the view generally adopted seems to be that of Percival, nevertheless it must be admitted that this remarkable phenomenon in phytopathology is not yet completely understood. Such being the case it seems that the methods of practical treatment advocated by various authors have hardly an adequate scientific foundation.

The external symptoms of Silver-leaf disease are obvious. As is well known, the leaves of the attacked trees show on their upper surfaces an ashen-grey colour giving a "silvered" appearance to the tree. The list of host trees is considerable including in particular nearly all the species of *Prunus*. The most important works on Silver-leaf disease are those of Percival (12), Güssow (3), Brooks (1) and Pickering. A complete bibliography and history of the whole question will be found in Güssow's work.

My contribution concerns the cytology of the attacked leaves. For the investigation I made use of the attacked and healthy plum leaves of the variety *Prunus domestica* var. "Victoria" which is a favourite in the English gardens because of its large and valuable fruit. Hand sections were made of some of the leaves; others were cut with the microtome.

Fleming's strong solution, which is very satisfactory for cytological work, was used as a fixing agent. In it the small pieces of the attacked and healthy leaves were exhausted by the water-pump and afterwards hardened in alcohol and embedded in paraffin. Many slides were made from material of three different ages, *i.e.* the middle of July, the end of September and the second half of October. By this means I perceived that the observed abnormal structures to be described later are not the consequences of autumn disorganisation; it can also be shown that we are not dealing here with artifacts. The sections were stained by different methods but all gave the same results. Mann's method (18, p. 735, Gram's iodine, eosin, toluidin blue), the inverse method (18, p. 809) by mordanting with potassium antimony tartrate, and the method of methyl-green with potassium hydroxide proved the best. Also Heidenhain's hæmatoxylin, safranin with anilin water, and gentian violet followed by eosin in clove oil were found satisfactory for the work.

I.

The anatomical structure of the attacked leaves differs from that of healthy ones. The thickness of the attacked leaves is somewhat greater than that of the healthy leaves. The turgor of the tissues in the former seems to be increased. Güssow (3, p. 393) states as the most striking anatomical phenomenon exhibited by "silvered" leaves, "dass sich die Zellen äusserst leicht von einander lösen und in dem Wasser des Präparates frei herumschwimmen. Nur mit äusserster Vorsicht gelingt es, kleine intakte Sektionen zu erhalten."

I also perceived that the cells of the spongy parenchyma fall apart very easily so that it is very difficult sometimes to obtain the sections intact. This happens only in the sections of fresh material, those cut from the thoroughly fixed and carefully embedded material were quite intact. And from such microtome sections the anatomical relations of the infected leaves could be observed quite readily.

The mesophyll of the leaves attacked by the Silver-leaf disease is thicker than the mesophyll of the healthy leaves or of the healthy parts of the attacked leaf. There are no striking changes in the length and arrangement of the palisade cells. But we notice in the spongy parenchyma that some cells are stimulated to a more intense growth in length; the intercellular spaces are in this tissue greater than in the healthy part of these leaves so that these changes remind us here and there of the structure of the gall in pear leaves caused by the mite *Eriophyes piri*, where the strong growth in length of all the mesophyll-cells together with the great enlargement of the intercellular spaces is the well-known symptom of this phytoptosis. But the cells of the spongy parenchyma in the silvering leaves do not remain together a long time; they fall asunder easily, as Güssow and Brooks state. Whole groups of the cells are disintegrated in this way which is the result of the dissolution of the middle lamellae. As is known a marked increase in size in the epidermal cells may also be observed. This is shown in the bulging of the walls of the epidermis towards the palisade cells. The volume of these cells is therefore greater. The tearing off of the epidermis from the palisade cells is very striking. Either the epidermis is simply detached and somewhat elevated so that a closed cavity is formed in this place, or the epidermis is quite torn asunder and the free portions are slightly lifted up from the palisades. Both this formation of cavities and the tearing of the epidermis are very common but not necessarily always present on all the areas attacked. A similar case was described by Miehe (vide 8, p. 478) as follows: "Epidermiszellen, die von *Synchytrium Taraxaci* infiziert worden sind, wuchern gegen das ihnen anliegende Mesophyll vor und drängen es beiseite u. dgl. m."

As for the cavities in the walls of epidermal cells which Percival (12) describes as a symptom of Silver-leaf disease I can state that I have looked for them in vain throughout an extensive series of preparations. In this respect my results agree with those of Güssow and Brooks.

The phenomenon of silvering of foliage which is the only external symptom of this disease is said to be due to the accumulation of air in the above mentioned subepidermal cavities, which interferes with the normal reflection of light. For instance it causes the white colour of the young *Bryum argenteum* leaves and of the white flower-petal, etc. I have had reason to doubt the adequacy of this theory and so I endeavoured to decide its value by the following experiment. The question is not so simple as it seems at first sight. The following experiment should dispel any doubts: the strongly attacked portion of a leaf was cut into small pieces which were then immersed in water in a vessel connected with an air-pump. Into the same vessel were put small healthy pieces of another shape—so as to distinguish them. These tissues were then completely injected with water. If the white colour is solely due to the air in the subepidermal cavities, it should disappear after injection. The leaves were cut in quite small pieces (about the average size of 2–3 mm. square) so that the water had easy access to the cavities. After the injection all the segments were carefully dried between filter paper and arranged in line, the attacked injected with the attacked non-injected and similarly the healthy injected with the healthy non-injected. Of course all the leaf-segments swelled up in the water. The white colour of the attacked segments was seen to have disappeared somewhat but not entirely so, for the green colour of the injected segments never equalled the bright green of the normal. The former always were a little dim. And moreover a piece of an attacked leaf loses its grey colour somewhat even without injection, viz. when it has merely been immersed for a short time in water. It became less and less till after about 25 minutes. It seems then that the existence of abnormal air spaces will not account completely for the silvered appearance of the leaves. It is very striking also that the spreading of the silvering always began first of all on and around the vascular bundles (veins) and from that it spread over the surface of the leaves. We can perceive in nature itself from the leaves only slightly attacked and also on looking at the photograph of infected twigs given by Güssow (3, p. 386) that the silvering of the leaves very often starts from the region of the veins.

On the above and the following grounds one must then be sceptical as to the "silvering" being really due to the accumulation of air in special subepidermal cavities: (*a*) it is a striking fact that the phenomenon of "silvering" spreads over the blade very often from the veins, above which the epidermis is very seldom separated; (*b*) the subepidermal cavities are not necessarily always present everywhere in the silvered leaves; (*c*) the contents of the epidermal cells and a certain disorganisation in the mesophyll (see below) can hardly fail to have an effect on the coloration of the foliage.

II.

Before dealing with the cytological changes in the mesophyll cells of the diseased leaves, one may consider a typical cell of the healthy mesophyll of a leaf of *Prunus domestica* var. "Victoria." Here we notice the simple normal relations of the cell content which can be observed in every healthy leaf. In the clear transparent cytoplasm lie the chloroplasts, ellipsoid in form, most commonly close to the cell-walls. They usually contain many starch grains which we can easily recognise in preparations stained by the "inverse" method. By other methods, however, the starch grains appear as structures shining through the thin transparent substance of the chloroplast. The nucleus stains intensely and is more or less spherical or oval and is often found in a somewhat central position in the cell. It usually contains one or two nucleoli and plenty of chromatin substance (Fig. 1).

If we compare with these the mesophyll cells of the "silvered" leaf—either the palisade or spongy parenchyma—we cannot be in doubt as to the difference between the cytological structure of the healthy and the infected tissues. There is often some difficulty in distinguishing the relations in the diseased mesophyll because the intensely stained chloroplasts make observation difficult. But careful study and suitable methods will reveal the true state of affairs.

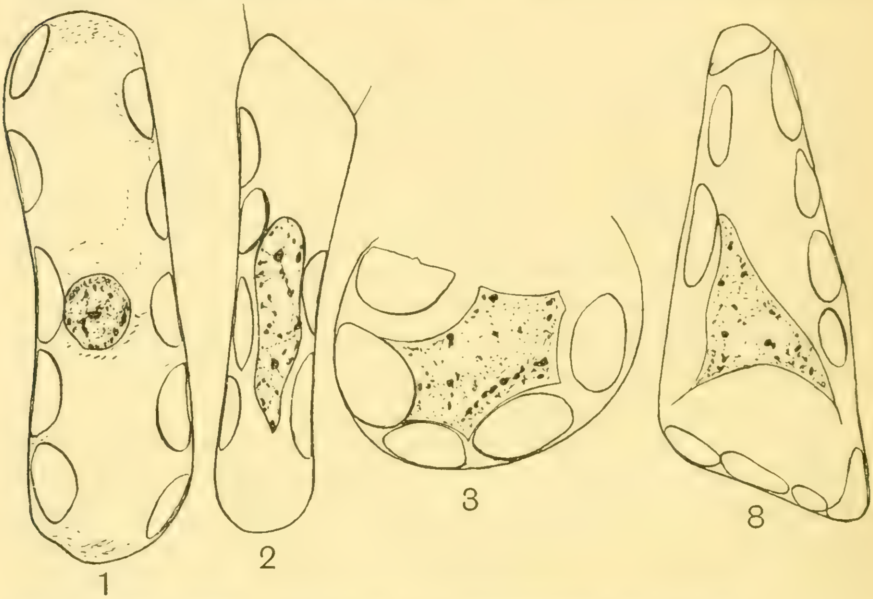
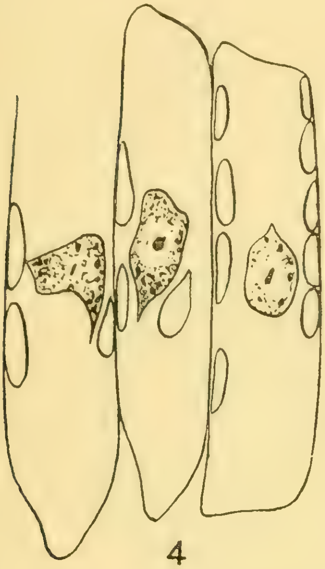


Fig. 1. Normal palisade cell from leaf of *Prunus domestica* var. "Victoria."
Figs. 2, 3, 8. Cells from an affected region of the same leaf, showing a hypertrophied nucleus (fig. 2), a nucleus with depressions corresponding to the surface of the chloroplasts (fig. 3), and a nucleus with filamentous projections (fig. 8).

I am unable to agree with Percival (12, p. 391) in his statement: "The peculiar light grey colour of the leaves is due to these air-filled spaces, and not to any alteration in the chloroplasts; the latter structures are of the same size and appearance as those in healthy leaves." On the contrary I have found changes in the nuclei, cytoplasm and chloroplasts of the affected mesophyll. In the same slide it is often possible to distinguish the healthy from the diseased part by staining alone. The cells with the disorganised content are wont to stain differently from those with the normal content, for instance the disorganised content of the preparations which are stained by Mann's method is obviously of a darker blue than the neighbouring healthy cells.

The nucleus. The changes in the nuclei which I was able to observe in the mesophyll cells of the diseased leaves are described according to their probable developmental stages in the living plant, in so far as it is possible to deduce these from stained preparations. First of all the nuclei become greatly elongated and hypertrophied, their volume is much increased so that they sometimes fill the cell-interior up to the chloroplasts, even touching them (Fig. 2). By this time the surface of the nuclei shows depressions corresponding often to the surface of the nearest chloroplasts (Fig. 3). The nuclei sometimes assume quite an irregular or amoeboid form (Fig. 4) producing lobes which seem to be directed between two neighbouring chloroplasts even when the nuclei do not fill the whole cell-interior (Fig. 5). In other cases the nuclei are seen to produce a long thin projection which is one of the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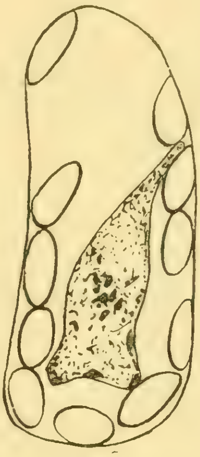
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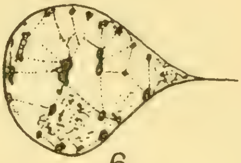
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Figs. 4, 5. Palisade cells with nuclei of amoeboid form.
Fig. 6. Nucleus from spongy parenchyma showing filamentous projection.

most remarkable features of disorganisation. Some nuclei send off two such fine projections. These thread-like projections are sent off either from the rounded nucleus or in most cases terminate one or more of the above-described lobes (Figs. 6, 7, 8).

In many other cases the elongated nucleus lies transversely across a palisade cell, giving the appearance of a transverse septum (Figs. 9, 10).

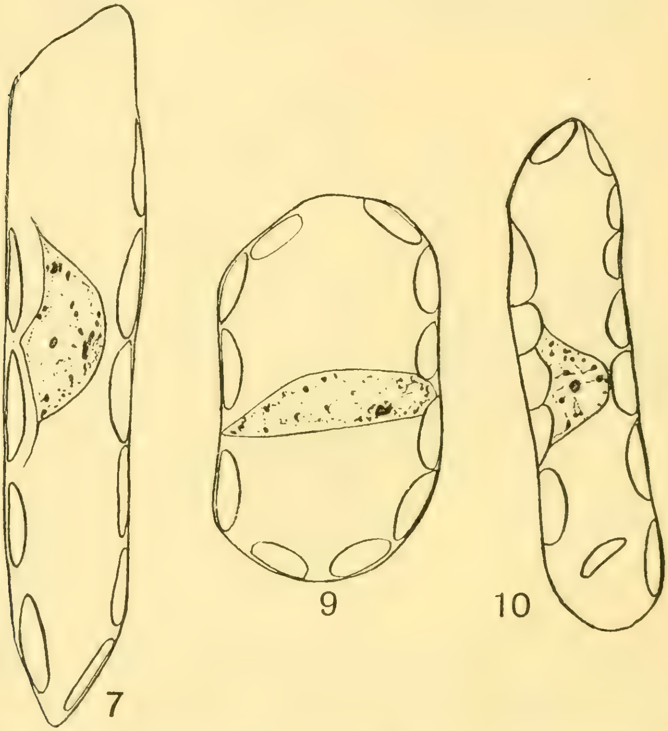
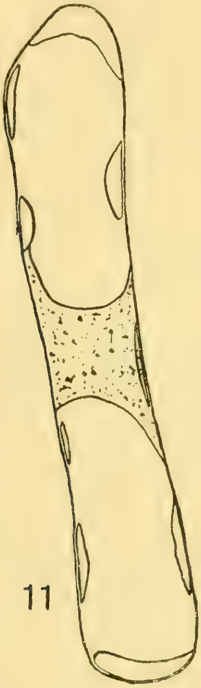


Fig. 7. Palisade cell showing nucleus with filamentous projections.
Figs. 9, 10. Palisade cells showing transverse orientation of nuclei.

And at the same time both ends of the nucleus spread somewhat laterally along the walls so that the nucleus, changed in this way, has two concave surfaces (Fig. 11). Sometimes this septum-like nucleus is of a considerable size so that it fills a third of a cell. The outlines of the septum-like nuclei become in some cases irregular (Fig. 12). While the septum-like nuclei appear in the palisade cells rather frequently, yet this is not the case in the spongy parenchyma. On the contrary, in the latter tissue the nuclei commonly show sharp pointed lobes as was above described. Probably the form which the disorganised nucleus takes may be dependent on the form of the cells (Figs. 13, 14).

As to the nucleolus, the normal spherical or only slightly deformed nucleus contains one or two easily distinguishable nucleoli. After subsequent disorganisation we cannot discern such obvious nucleoli in the nuclei.

In addition to modifications of form of the nuclei marked changes take place in the chromatin. The normal nucleus contains a conspicuous network with numerous intensely stained chromatin granules. But as it disorganises the nucleus always contains fewer and fewer granules. There is strong evidence (see below) for the view that these grains sometimes wander out from the nucleus into the cytoplasm. Cases were found which were a distinct proof of this. The chromatin manifests a tendency to accumulate on the periphery of the nucleus. It is possible that the grains move centrifugally and press against the nuclear membrane, for we notice the chromatin grains accumulated close to



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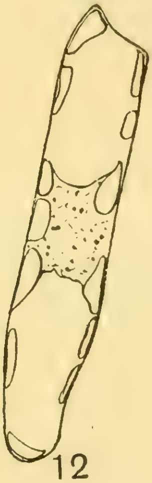

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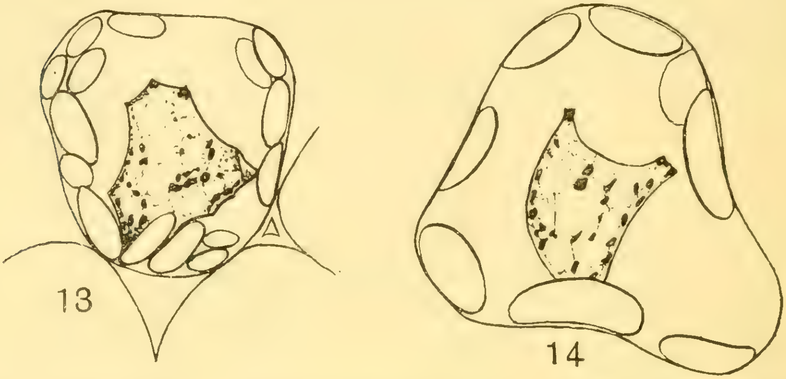
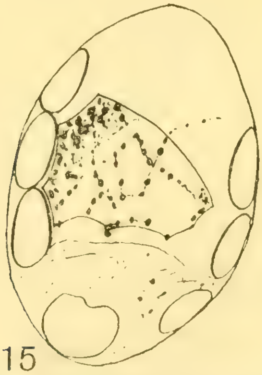
Fig. 11. Palisade cell showing septum-like nucleus.
Fig. 12. Palisade cell showing septum-like nucleus with irregular outlines.

the wall of the deformed nucleus (Figs. 13, 14). Apparently the different forms of the disorganised nuclei are connected with the wandering out of the chromatin grains. Here and there we can observe that the chromatin grains are outside the nucleus (Figs. 15, 16), and what is more a whole line of several chromatin grains lies, in a few cases, outside of the nuclear substance (Fig. 15). In such a case the nuclear membrane seems to be very thin; or it disappears entirely in some places, but this is a rare occurrence. Another reason for the view that chromatin grains migrate from the disorganised nucleus is the occurrence in the palisade parenchyma of disorganised nuclei which are without



Figs. 13, 14. Cells from spongy parenchyma showing nuclei with sharp-pointed lobes.



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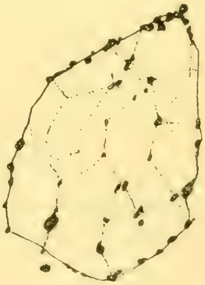

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Fig. 15. Cell from spongy parenchyma showing chromatin grains outside the nucleus.
Fig. 16. Nucleus from palisade cell showing chromatin grains accumulated at the periphery.

chromatin grains (Fig. 17). The shrivelled remains—probably dead—of the septum-like nucleus appear as a homogeneous substance (Fig. 17) which stains uniformly and somewhat differently from the nuclear substance in the earlier stages. The migration of the chromatin from the nucleus was described in *Nematode*-galls by Nemec (11, pp. 171, 483) and by Guttenberg (vide 7, p. 202) in the galls of *Ustilago maydis*.

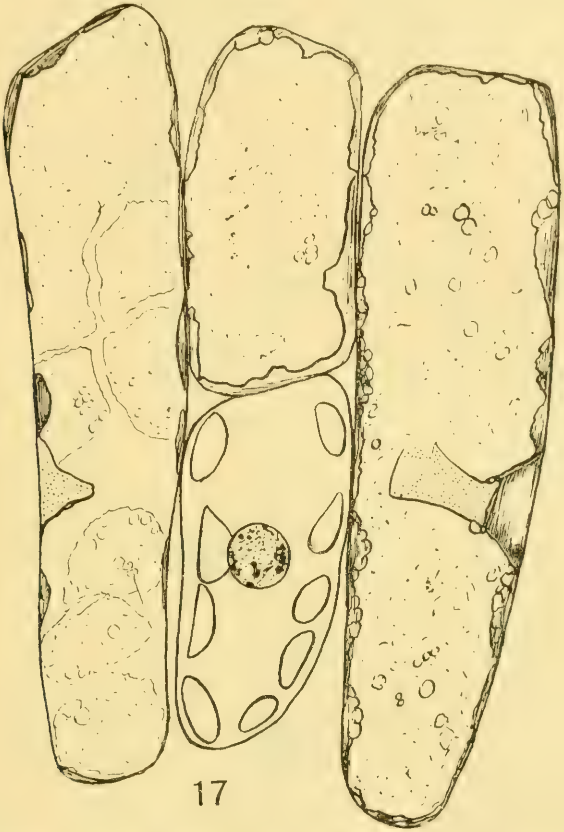

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Fig. 17 Cells from palisade tissue. Three of them showing disorganised content; chloroplasts destroyed and mostly vacuolised; remains of nuclei (in the long cells on each side) homogeneous and without chromatin granules. The fourth cell, the short one in the middle below, is normal.

It is doubtful whether the phenomenon here has any definite relation to mitochondria as they are conceived by Arnoldi[1] (vide 15, p. 707). For the chromatin grains in the cytoplasm just outside of the nucleus very seldom remain as permanent structures in the diseased *Prunus* leaves. They are most probably very quickly dissolved in the cytoplasm, as was found in the *Nematode*-galls by Nemec. Such an outward movement of chromatin grains away from the nucleus is hardly a normal process; it may be a symptom of pathological conditions in the cell, just as is assumed for the *Nematode*-galls by Nemec, who says: "In den Riesenzellen der *Heterodera*-gallen bei *Washingtonia robusta* treten zwar in einem bestimmten Stadium regelmässig fast alle Chromatinkörner aus dem Kern heraus, aber die Kerne degenerieren hierauf, und es scheint daher, dass es sich um einen pathologischen Vorgang handelt" (11, p. 483).

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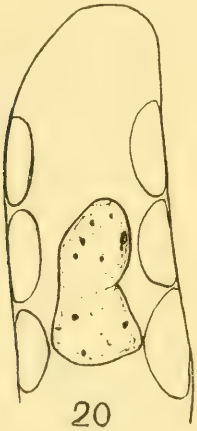
[1] Arnoldi states that the mitochondrium is the same as the chromidium, viz. that the mitochondrium is of nuclear nature. But this view has very few adherents to-day.



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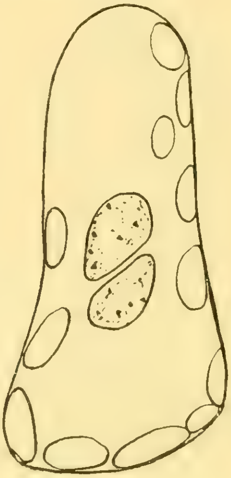

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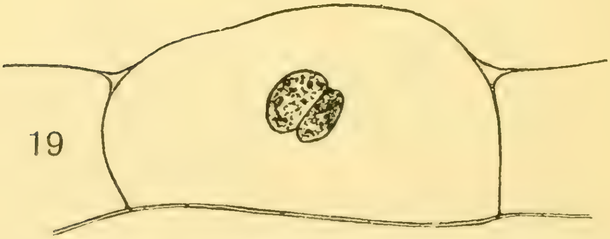

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Figs. 18, 19. Binucleate cells from palisade tissue and upper epidermis respectively.
Fig. 20. Part of palisade cell showing constricted nucleus.

In a few cells I have found in the silvered leaves nuclear "fragmentation" or at least a tendency to it. In several palisade cells and in a single epidermal cell two nuclei occur in close proximity to each other (Figs. 18, 19). Or the elongated nucleus shows a simple constriction (Fig. 20) which may be the beginning of amitosis. The direct division in the plant tissue is, according to the generally accepted view, a characteristic of degeneration or of pathological conditions. I did not succeed in following the fate of the amitotically divided nuclei. Probably they disorganise in the same manner as the nuclei in the uninucleate cells of the diseased area, since in one binucleate palisade cell at least one nucleus showed obvious traces of early disorganisation. In some other attacked cells—as was described above—it can be definitely established that the nucleus becomes more and more deformed, disorganised and finally dies (Fig. 17).

The chloroplasts. In the diseased area the chloroplasts show remarkable diversity of structure and form, being often very irregular and sometimes destroyed. The beginning of their disorganisation appears as a corrosion of the surface. Their volume is consequently markedly reduced in size and thus in optical section they appear thinner and thinner (Fig. 17). The unusual irregular outlines can be easily observed on the chloroplasts as seen in surface view in a cell uncut by the microtome-knife.

In the strongly affected region the chloroplasts finally appear as very thin scale-like structures, sometimes hardly distinguishable and lying close to the cell walls (Fig. 17). By this time the cell is very often filled with a curious granular substance. In such cells we do not notice any remains of the nuclei. There is no likelihood that the nuclei were removed by the microtome-knife because whole rows of the destroyed cells in such a diseased area do not contain nuclei. Starch grains were never found in the deformed chloroplasts.

The disorganisation does not progress everywhere in the cells in the same way. In some cells the nucleus appears almost normal or only somewhat hypertrophied, while the chloroplasts may be in an advanced stage of disorganisation. On the contrary we notice cells where the partly disorganised nucleus is found in the cell along with healthy chloroplasts. Yet in most cases the disorganisation progresses equally in the two.

There can be no question of the changes described being of the nature of artifacts, for in the immediate vicinity of the disorganised cells we can readily observe others with quite healthy contents (Fig. 17). Also the results of autumn changes are here excluded, because all the described cytological modifications appear in leaves fixed not only in October and September but also in those fixed in July.

The cytoplasm forms a peripheral layer in the healthy mesophyll cells of *Prunus* leaves and is thin and clear, but in affected cells it is clouded. The cell contents of the diseased area are very striking because of a uniformly stained, granular deposit. This fine granulation occurs in the palisade and spongy parenchyma and epidermis alike and sometimes in the intercellular spaces. It is always in the silvered "Victoria" plum leaves a certain indication of a diseased area. It was never found in the cells with entirely normal content. An attempt to determine the nature of this substance by microchemical methods was not very successful. The tests for sugar (Fehling's solution) and for protein (Millon's reagent) gave negative results. Usually the test for tannin (ferric chloride, potassium bichromate) gave negative results also, although occasionally very slight reactions were obtained.

It would appear that the vascular bundles (veins) play a certain part in spreading disorganisation in the mesophyll. It can often be noticed in the sections that the vascular bundles limit the diseased area; cases were observed where on one side of vascular bundle the tissue was more or less profoundly altered, whilst on the other side the cells did not show any symptoms of attack. It is possible that this fact is connected with the observation previously mentioned of the spreading of silvering from the veins.

III.

Discussions and Conclusions.

The facts above described show clearly that the changes occurring in "silvered" leaves of the plum are not confined to the development of air spaces and the separation of cells but are far more profound than was supposed. There is no doubt that in the diseased leaves markedly abnormal physiological conditions exist.

It is true that some of the phenomena described take place only in pathological tissue, while others are known occasionally in healthy tissue also. For let us consider briefly a few features of the silvered leaves which are similar to those in healthy tissue. The abnormal increase in size of the nucleus is—to judge by the manner of its occurrence—connected with the increase of metabolism just as it is for instance in the large nuclei in the glands of animals or in healthy tissue of the seaweed *Antithamnion* (Schiller, 14), where the surface of the nuclei is much increased in size by enlargement and change of form or development of lobes. In the diseased *Prunus* leaves the hypertrophy of the nuclei means the beginning of a reaction and we cannot decide whether it is response to the increased metabolism only or to the influence of a toxic substance.

Concerning the amitotic mode of division the most recent view is that it is not a phenomenon that appears in healthy tissues unless the latter are old (*e.g.* the well-known case of amitosis in the old cells of *Chara*) or degenerate. Many cases of apparent amitosis have been explained as modified stages of mitosis (Nemec, 10). There are, indeed, authors who state that even in wound tissue the mitotic mode of division regularly takes place (Strasburger, 19, p. 22). On the contrary we often meet with amitosis in the hypertrophied tissue of galls although even in the latter karyokinesis occurs (Küster, 7, p. 200). Amitosis was observed by Guttenberg in *Capsella bursa-pastoris* after infection by *Cystopus* (Albugo) *candidus*, and by Shibata in *Podocarpus*-galls, etc. (Küster, 7, p. 200). Amitotic mode of division—so far as is known —is never followed by cell division, so that bi- or multinucleate cells result. The few cases of amitosis in the silvered *Prunus* leaves are what one would expect in the organs attacked by such a serious disease ("der Milchglanz sei ein absolut sicherer Vorläufer des Todes eines Zweiges," Sorauer, 17, p. 285), where the conditions leading to hypertrophy prevail. Amitosis here is evidently similar to amitosis in galls. The few binucleate palisade cells observed are probably in respect of their origin the result of amitosis.

Some modifications in the cell and nucleus resembling the condition found in *Prunus* leaves have been often brought about by artificial methods. Thus, for example, twenty years ago Klemm (5) investigated the phenomena of disorganisation in the hairs of *Urtica*, *Momordica*, *Tradescantia* and *Trianea* caused by abnormal conditions of temperature, light, electrical action and the influence of acids. In his experiments marked changes in the protoplast appeared particularly in response to electrical action. The nuclei became elongated or in other ways deformed and finally destroyed. The granular appearance of the nuclei changes to a homogeneous ("ein glasiges homogenes Aussehen," p. 688) and the nucleus later collapses completely just as happens in some cases in the silvered leaves (Fig. 17). Otherwise in Klemm's experiments "der Kern erleidet bei der Desorganisation der Zelle allgemein wenig sichtbare Veränderungen" (p. 686). Several years later Nemec (10) obtained interesting modifications of nuclei by the influence of chloralhydrate, some cases of which resemble these in the diseased *Prunus* leaves.

In the production of abnormalities in the protoplast various other factors have been found to play a certain part. The important literature on this subject is contained in the work of Reynolds (13).

In the cases cited various modifications in the protoplast were attained artificially, viz. by the introduction of a definite external agent. When such a factor induces marked degeneration—as *e.g.* in the electrical radiation experiments of Klemm—then we can look upon it as a strong stimulus in the life of the cell. The pathological factors (vapours of chloroform, electrical radiation, etc.) acted *directly from the immediate vicinity of altered cells.* If we notice in the *Prunus* leaves changes of a like magnitude in the cells, then we can judge in an analogous way of a strong stimulus which probably also is acting somewhere in the immediate vicinity. We may judge of it even more inasmuch as the changes observed in the mesophyll cells of *Prunus* remind us strikingly of those which take place in the various galls, so that it is permissible to compare a leaf attacked by Silver-leaf disease with a gall, even according to the definition of Küster (7, p. 2).

Within the last few years cytological investigations have been carried out on the pathological reactions of the diseased plant. The important literature of this subject is to be found in Küster (7, pp. 198–205) and in the recent work of Reynolds (13). The attention of Reynolds was directed toward the reactions of leaf tissue to fungal invasion in the various phanerogamic plants. *Zea Mays* parasitised by *Ustilago Maydis*, *Pirus malus* by *Gymnosporangium* sp. and *Viola cucullata* by *Puccinia Violae* are among the cases examined. There are also included some host plants (*Panicum, Smilax*) upon which the cause of the disease is not clear but is certainly of fungal nature. It cannot be doubted that many changes in the *Prunus* leaves attacked by Silver-leaf disease remind us of those described by Reynolds. This author also states that the nuclei in the parasitised leaf tissue are, as a rule, more or less deformed and enlarged, varying from globular to pear- or even crescent-shaped; that the chloroplasts may be affected, at least in shape and size (*Viola* parasitised by *Puccinia*) and the chlorophyll may disappear (*e.g. Panicum, Potentilla* by rust, etc); that the cells before collapse are filled with a granular yellowish substance (*Gaylussacia baccata, Zea* in epidermis) and that the pathological tissue is sometimes entirely destroyed and killed (*Smilax, Castanea*). All these modifications brought about in the cells by influence of a parasite from close proximity *are quite comparable to the changes of the cytological elements in the case of Silver-leaf disease.* The affected plants which formed the subject of investigations by Reynolds are to be considered as galls (viz. *mycocecidia*) according to the terminology of Küster.

Changes similar to those which occur in *Prunus* leaves are well-known phenomena also in other galls, whether *mycocecidia* or *zoocecidia*. Enormous hypertrophy of the nuclei may occur in all galls and may attain sometimes unusual dimensions. The nuclei of the host plant under the influence of *Synchytrium* (Guttenberg, 4, p. 438) may become 250 times the volume of the normal nuclei. "An enlargement of the nucleus often to double the normal size and often a change of shape to spindle form" was observed also by E. F. Smith (16, II. p. 92) in plant organs attacked by bacterial disease. Also "septum-like" nuclei similar to those which occur in diseased *Prunus* leaves were established by Guttenberg in *Alnus incana* attacked by *Exoascus amentorum* (after Küster 7, p. 202). The pathological nature of the amitosis and also the forcing of chromatin grains toward the periphery of the nucleus, have already been pointed out.

Not only the cytological characteristics but even the anatomical features of the silvered leaves investigated show a cecidiological nature. The study of histogenesis of galls has shown that the abnormal growth of host cells is a symptom of all galls. In the case of the Silver-leaf disease the hypertrophy of the mesophyll is perhaps not very marked, but yet it is quite obvious from a comparison of healthy and affected mesophyll. A similar hypertrophy of the mesophyll was noticed by Kusano in the leaves of *Vicia unijuga* attacked by *Olpidium Viciae* Kus.: "The mesophyll of the diseased spot is hypertrophied with the enlargement and the increasing number of cells" (6, p. 177). The falling asunder of mesophyll cells which has been previously noted in the silvering leaves by all authors appears also in the leaf-gall of *Oligotrophus bursarius* on *Glechoma* (Küster, 7, p. 197). Moreover the epidermis ruptures under the influence of *Synchytrium Taraxaci*, as already stated.

If we review the comparisons just mentioned we must admit that *the affected tissue in the case of Silver-leaf behaves as a parasitised tissue, and on structural grounds belongs to the category of gall-tissue, using gall in the wider sense of the word.* It is true that there is no characteristic common to all galls, but every pathological feature of the Silver-leaf disease finds a parallel in some gall.

Although the study of the etiology of Silver-leaf disease lies beyond the scope of this contribution yet one etiological deduction was of necessity forced upon one during this investigation. According to the investigations of Percival, Pickering, Güssow, Brooks (cited above) the basidiomycete *Stereum purpureum* is the cause of Silver-leaf disease as was indicated by successful inoculation experiments. In all these experiments the inoculation of the tree by *Stereum* was followed by the external symptom of the silvering of the foliage, but the fungous hyphae were never found in the leaves. I observed also that there is no trace of any hyphae in the silvered *Prunus domestica* leaves. How are we therefore to account for these remarkable phenomena of disorganisation in the plum-leaf? If the mycelium of *Stereum* existed in the leaf, then the changes described would be quite comprehensible. The diseased leaf with its abnormal internal conditions would be classed without any hesitation among the galls, as are the cases described by Reynolds. It is true that a parasite can produce sometimes marked changes in the nucleus even when it is not in immediate contact with the latter; but in the case of Silver-leaf disease the mycelium of *Stereum* when present is far distant. For the *Stereum* spreads—as it was established by the authors mentioned—in the wood elements of root and stem but never in the leaf nor its petiole. Since the attacked leaf behaves exactly as a gall tissue parasitised by an organism living in the tissues, it is difficult to believe a mycelium so remote could be the cause of such changes of nuclei, chloroplasts and cytoplasm.

Not only the changes of the protoplast, but also the spontaneous maceration of the mesophyll tissue is difficult to explain as a result of the action of a distant mycelium. The latter phenomenon, common in the silvered leaves, reminds us somewhat of the results in Richter's (after Küster's *Aufgaben*, etc. 8, p. 462) experiments with narcotics (vapours of camphor), where a similar maceration of tissue was obtained. The falling asunder of cells, as it is known, may be caused in plants parasitised by an organism in these ways; either the parasitic hyphae penetrate and dissolve the cell walls by cytase or the host cells themselves produce the enzyme under the influence of the parasite. If *Stereum purpureum* is the direct cause of the maceration of the leaves, as has been suggested, then a fungous enzyme must be carried up by the vascular bundles from the lower parts of the host. It is unlikely that an enzyme of the nature of a cytase would be so carried since it would cause the destruction of the vascular strands and other tissues *en route*. It is much more probable that some toxin is secreted in the leaves which causes the changes which have been described. The action on the middle lamella of the leaf cells is probably due to an enzyme secreted by the cells as result of poisoning, *i.e.* is an autolytic action.

It is, however, not at all certain that *Stereum purpureum* is really responsible for the Silver-leaf disease. Indeed we find in the literature concerning the subject of this disease a considerable amount of doubt. At the beginning of this paper various views respecting the etiology of this disease were pointed out. Massee (9, p. 66) has failed to find any hyphae in affected plants. Even Brooks has not found Stereum in all cases of this disease. This author examined some silvered plum seedlings which were growing from seeds obtained from healthy trees, and he states: "The silvering was strictly comparable with that which occurs in adult trees. The epidermis was partly free from the underlying palisade tissue and on trying to cut sections of the leaf there was a decided tendency for the mesophyll cells to fall asunder one from the other. There was no evidence of fungal attack in either leaf, stem, or root. As already stated, the seedlings began to recover when given more room in which to grow and upon examining them in August, I saw that the recovery of the foliage to its normal appearance was well advanced. I came to the conclusion that in such a case as this the phenomenon of Silver-leaf was not caused by *Stereum purpureum*" (Brooks, 1, p. 291). Or on the contrary: "...cases have been already mentioned in which Silver-leaf has not resulted although *Stereum purpureum* has made considerable progress in the tissues. I have seen apple and beech trees which have been killed by *Stereum purpureum* in all probability, but with which the phenomenon of Silver-leaf has not been associated" (Brooks, 1, p. 307). In the light of this evidence it is not possible to believe that *Stereum purpureum* is the sole cause of the Silver-leaf disease! Perhaps the inoculation experiments hitherto carried on have been too few or possibly they are not yet complete enough to decide the relation of *Stereum* to this disease. In Bohemia the same external phenomenon, viz. the silvering of foliage, has appeared frequently in recent years on the leaves of sugar beet (*Beta vulgaris*). Of course we must not draw conclusions here from the similarity of the external symptoms, nevertheless it seems to me that the study of this affection, which of course is not caused by *Stereum*, may possibly explain some obscure points connected with the Silver-leaf disease in trees.

Further studies on the cytological changes occurring in diseased plum leaves would be of value. The changes found in leaves in the spring should be studied. Besides the phenomena above described I found bacteria in the diseased plum leaves; thus occasionally in the cells of the parenchyma bordering on the vascular bundles or in the epidermal cells. I succeeded in staining them by the method of E. F. Smith (16, III. pp. 129, 914). These bacteria may here be of a secondary nature; nevertheless their appearance is a promising subject for further study[1]. I hope to continue along this line later but for the time being I am not able to extend the work.

The question of the Silver-leaf disease is of economic importance especially in England, as the losses caused by it reach a considerable amount. It is not so in Bohemia, where this disease appears frequently, but its virulence is not yet so marked.

The investigation here recorded was carried out at the suggestion and under the direction of Professor V. H. Blackman, and I wish to acknowledge my indebtedness to him for his valuable suggestions and for the interest with which he followed my work. I desire also to thank Mr W. Brown, M.A., of the Imperial College of Science and Technology, London, and Dr Arthur H. Graves of New Haven, Conn. U.S.A., for kindly editing the English text.

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[1] I regret that the work of Blackmore (quoted in the work of Güssow) is not accessible to me. This author is of the opinion that bacteria are the cause of this disease.

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NOTES ON SOME HYMENOPTEROUS PARASITES BRED FROM THE PUPAE OF *CHORTOPHILA BRASSICAE* BOUCHÉ, AND *ACIDIA HERACLEI* L.

By J. T. WADSWORTH,

Research Assistant, Dept. of Agricultural Entomology,
University of Manchester.

During the course of an investigation of the life-histories of parasites which attack soil insects, and more particularly of the life-history of *Aleochara bilineata*, Gyll., a Staphylinid whose larvae infest the pupae of the cabbage-root fly *Chortophila brassicae*, the following parasites were reared:

A. From the pupae of *Chortophila brassicae*:
  1. *Phygadeuon fumator*, Grav.
  2. *Atractodes tenebricosus*, Grav. (*vestalis*, Hal.).
  3. *Cothonaspis* (*Eucoila*) *rapae*, Westw.

B. From the pupae of the Celery-fly (*Acidia heraclei*).
  4. *Hemiteles crassicornis*, Grav. (= ? *subzonatus*, Grav.).
  5. *Adelura apii*, Curtis.

As the species numbered 1 to 4 do not appear to have been recorded hitherto from these dipterous hosts, the following remarks on them may be of interest.

1. *Phygadeuon fumator*. Morley (*Ichneum. Brit.* vol. II. pp. 98–99) states that this is one of the most abundant of all British insects, and considering its prevalency, it has very rarely been bred. Its previously recorded hosts are: *Mamestra brassicae* (the cabbage moth), *Emphytus serotinus* (a saw-fly), and a dipterous puparium found in carrion. Morley comments on these records as follows: "I suspect it of preying mainly on Anthomyiid diptera": and he informs me (*in lit.*) that he considers the records of the first two hosts to be erroneous. It is therefore interesting to find his suspicion confirmed. From several hundred puparia of the cabbage-root maggot I have obtained only one specimen, a female,

which emerged July 26th, 1914. The host's puparium was jet black and readily distinguishable from the brown unparasitised pupae.

2. *Atractodes tenebricosus.* This species has been recorded from a great number of localities in Great Britain and Ireland; it is probably ubiquitous. Morley (*loc. cit.* p. 247) remarks that he can find no record of its parasitism. From 506 puparia only two specimens were obtained. One female emerged at the end of March, 1914, and one male on May 2nd, 1914.

3. *Cothonaspis rapae* was obtained in abundance from puparia of *C. brassicae.* This Cynipid was first recorded and described by Westwood (*Mag. of Nat. Hist.* vol. VIII. 1835, pp. 171–9) from some examples sent to him for identification; they were obtained from turnips on which dipterous larvae were feeding. Westwood, however, believed that the Cynipid larvae also fed on the turnips and that the Cynipid and the dipteron had no further association with each other. In the light of our present knowledge we now know that Westwood's supposition was based on insufficient data; so few Cynipids, however, were then known to be parasitic that his mistake was quite excusable. Concerning this species Cameron (*Monog. Brit. Phyt. Hymenopt.* vol. III. p. 210) remarks, "bred by Westwood from the tumours on turnips formed by *Ocyptera brassicaria.*" There is no mention made of *Ocyptera* in Westwood's article quoted previously, and I have not attempted to trace the source of Cameron's statement[1], but there appears to be some mistake with regard to it, as the Ocypteridae are parasitic diptera attacking, so far as known, Orthoptera, Hemiptera, Coleoptera, and Lepidoptera (Townsend, C. H. T., *Insect Life*, vol. VI. 1894, p. 201). It is therefore very improbable that *Ocyptera brassicaria* could form tumours or galls on turnips. The cabbage-root maggot, however, attacks turnips as well as members of the cabbage family, and I believe the specimens described by Westwood probably emerged from the dipterous puparia whose larvae had been feeding on the turnips. The statement of the observer who sent the insects for identification, that the larvae found feeding on the turnips were "exactly like those in the knobs of cabbages," lends support to the above explanation. Westwood figures a pupa obtained from one of the larvae and, so far as one may judge, it was a pupa of *Chortophila brassicae.* As the Eucoilinae are recorded as attacking Tachinidae, the possibility of *C. rapae* having been bred from *O. brassicaria* is, however, not excluded. There are at least two

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[1] Probably specimens so labelled were among Westwood's 1833 types, when these were examined by Cameron in 1888 (cf. *Ent. Mo. Mag.* XXIV. p. 209).—Claude Morley.

generations of *C. rapae* produced each year. One generation emerges in April and May, and the majority of the second generation in August. A few individuals, however, emerge during the later months of the year. I have a record of one specimen which emerged near the end of December, 1914.

4. Of *Hemiteles crassicornis*, one of the two species of parasites obtained from the celery-fly puparia, Morley (*loc. cit.* p. 141) notes that it is doubtless common, and had not then (1907) been bred. I obtained it from puparia collected in October, 1913, in the garden of Mr H. Bury, High Lane, Cheshire, together with—

5. *Adelura apii*, a Braconid, which, according to Marshall (*Monog. of Brit. Bracon.* Pt. VI. pp. 367–8) has been frequently reared from *Acidia heraclei*. Mr Bury sent me thirty-eight celery-fly puparia, and of these thirty-four were parasitised. Three examples of *A. apii* were observed on December 6th, 1913. No further parasites were seen until April 30th, 1914; between this date and May 14th seven *H. crassicornis* emerged, and on August 11th another individual of this species was obtained. Of the eight *Hemiteles* obtained four were females and four males.

In 1914 the first *Adelura* was recorded on May 16th, and subsequently they emerged at intervals of two or three days until June 23rd. Between these two dates 16 specimens were reared. No further examples were noted until August 11th, when another individual was obtained. Twenty *A. apii* were obtained altogether and of these seven were females and nine males; the sex of four individuals was not determined. Two of the celery-fly puparia were opened in January, 1914; they contained fully fed parasitic larvae, and only four celery-flies, which emerged at various times, were obtained altogether. An examination of the remaining puparia was made and four were found to contain dead parasites, of which one was *A. apii*.

From thirty-eight puparia of *Acidia heraclei* were obtained:

4	*Acidia heraclei* (adult flies).
8	*Hemiteles crassicornis.*
20	*Adelura apii.*
2	parasitic larvae.
4	dead parasites which failed to emerge, one of which was *A. apii.*
38	Total.

As may be seen, the number of infected pupae was very large. The high degree of parasitism is rather remarkable, but the number of pupae from which these records were made is too small to admit of any general conclusions being based on the results obtained. It is, however, worthy of note that last year very few celery plants in Mr Bury's garden were found to be affected by celery-fly larvae, and there may possibly be some relationship between this fact and the high degree of parasitism of the pupae from the last brood of celery-flies of the previous year (1913).

A larger number of celery-fly larvae and pupae were collected in the autumn of 1914 at the Agricultural College, Holmes Chapel, Cheshire, and in the neighbourhood of Northenden, Cheshire, with the object of obtaining results on a larger scale. These larvae and pupae were also found to be heavily parasitised. The complete results of the investigation will not be obtainable, however, until August or September, 1915, owing to the period of emergence of the parasites extending over so many months of the year.

I wish to express my thanks to Mr Claude Morley for naming the Ichneumons (1, 2 and 4), to Professor Dr J. J. Kieffer, of Bitsch, for identifying the Cynipid (3), and to Mr G. T. Lyle, of Brockenhurst for confirming my identification of the Braconid (5).

NOTE ON AMERICAN GOOSEBERRY MILDEW.

By M. A. BAILEY, B.A.

John Innes Horticultural Institution.

During the last four years a large number of seedling gooseberries have been raised here each season in the course of an experimental study of the inheritance of disease-resistance and other characters in this group*.

Many of these seedlings are crosses between English and American types and, as such, are either partly or wholly immune to American Gooseberry Mildew, but the majority were obtained by the self-pollination of various English varieties. The following observations on the incidence of disease refer solely to the latter class.

The first batch of seedlings raised—about a thousand plants—were pricked out in the open (Plot *A*) on May 6th, 1912. They remained free from disease till about the end of June. On August 25th and subsequent days a careful examination of the individual plants was made, and the intensity of infection and relative position of the bushes noted. This examination showed that about 40 % of the plants were infected with mildew, the intensity of the attack varying from "slight" to "very severe." Infected bushes were found in all parts of the plot, and a tendency to occur in groups suggested that the primary infection was sporadic but widespread in its distribution.

The manner in which this primary infection took place is not known but it is possible that it was connected with the presence during the previous year of two infected gooseberry bushes in a rather distant portion of the grounds. These two bushes were destroyed in September, 1911, but by that time some of the perithecia would have already fallen, and ascospores from these may have produced the infection in 1912.

In cases of slight infection the mildew was found chiefly on the underside of the young leaves, and frequently occurred high up on the bush. The more heavily infected bushes showed mildew on the young shoots, and an examination of these shoots made at a subsequent date showed perithecia present amongst the mycelium, but the condition of the ascospores in these perithecia was not ascertained.

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* In this work I have had the collaboration first of Mr W. O Backhouse and, subsequently, of Mr J. W. Lesley.

When the examination of the bushes had been completed, they were sprayed with Liver of Sulphur with the idea of keeping the disease under control, though this wash has since been proved by Professor Salmon to have little or no effect in the case of this particular mildew.

On November 6th all the bushes were transplanted to another plot (Plot *D*) situated about 50 yards from Plot *A* and separated from it by a low hedge. As it was desired to make observations on the natural habit of growth of these seedlings, they were allowed to remain *entirely unpruned*.

The following year (1913) a new batch of seedlings was pricked out during June on a portion of Plot *A*, which had been occupied by the infected seedlings of the previous year.

The summer of 1913 appeared to be unfavourable to the growth of the mildew, and a relatively slight, though more or less general, infection was recorded on the seedlings of Plot *A*. The season was a very dry one, and this probably had some effect, if only through the more rapid ripening of the young wood. The plants in Plot *D*, many of which, as recorded above, had been heavily infected during the previous year, remained entirely free from mildew, with the exception of one or two bushes—less than 0·5 %—which showed very slight traces of disease.

During the winter the plants received rather light pruning.

In May, 1914, the remaining portion of Plot *A* was filled up with newly raised seedlings, those of the previous year being allowed to remain in their places.

Mildew reappeared during the summer in Plot *A*, and in August it was found that almost every one of the one-year-old plants was more or less heavily infected, as also were all those of the current year, which had made sufficient growth. As in the previous year, the plants in Plot *D* remained practically free from mildew, despite the fact that they made a lot of young growth, of the kind which is looked upon as being specially susceptible to attack.

To account for the apparent lack of infection during the summers of 1913 and 1914 of the plants in Plot *D* by oidia from the infected plants in Plot *A*, I can only suggest that the presence of the low hedge between the two plots coupled with the fact that Plot *D* lies more or less to windward of Plot *A* (*D* lies south of *A* and the prevalent wind is S.W.) has been sufficient to prevent the transportation by wind of all but a relatively few spores.

Mr Rogers, in a note in the January number of this *Journal*, refers to a case in which all the visibly affected wood of diseased bushes was removed, and the bushes transplanted to uninfected ground, with the result that they were free from disease in the following year. In view of the occurrences described above, it seems difficult to resist the conclusion that it was the transplantation and not the pruning which was the important factor in the recovery of these bushes, and it is even possible that they might have remained free if the pruning had been omitted.

Salmon has recorded[1] that if shoots bearing the winter stage of the mildew are removed at the beginning of August and gently tapped over a piece of paper, dozens of perithecia, ripe and in excellent condition, fall from the mycelium.

The above observations tend to confirm his view that the majority of the perithecia fall from the meshes of the mycelium in the late summer and autumn, and that of those which remain throughout the winter very few are viable.

On the other hand, Salmon, in a paper read at the meeting of the Association of Economic Biologists, 1914, refers to the frequency with which the disease reappears first on young berries on the *upper* branches, and to account for this he suggests that in these cases the reinfection has originated from perithecia which had lodged in crevices in the bark or between bud scales, etc. Though, in my experiments, the first raised seedling gooseberries were only about nine months old when moved to their new quarters, the majority had already made considerable growth. In many cases the bark had begun to show longitudinal cracks or even slight flaking, and the plants were, of course, plentifully supplied with bud scales. Also, if the primary reinfection originates from perithecia entangled in this manner, it does not seem clear why the lower branches should not be affected to an equal degree.

It will be seen above that in the case of the first batch of seedlings raised there was a tendency for the mildew to appear more particularly on leaves high up on the bushes, and it seems possible that the explanation of this and of the case described by Mr Salmon may lie in the different atmospheric conditions which obtain at the top and at the bottom of a bush, when its leaves are sufficiently developed. Spores which alight near the top of a bush must be subjected to greater ranges of temperature and greater degrees of desiccation than those which fall on to leaves growing lower down.

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[1] *Journal of Agr. Sci.*, vol. VI. p. 2, May, 1914.

It is possible that these or some similar factors may have a favourable influence on the germination of the spores, thus producing the effect described.

The favourable effect of cooling on the germination of spores of *Cystopus candidus* has been shown very clearly by Melhus[1], and Eriksson and Henning[2], working on the Uredineae, found that aecidiospores which had been placed on ice for a time gave a much higher percentage of germination than those which had been left throughout at the temperature of the room.

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[1] Wisconsin Agl. Expt. Station *28th Ann. Rept.,* 1911.

[2] *Die Getreideroste,* p. 73.

THE EFFECT OF VARIOUS CHEMICALS ON BLOW-FLY.

By W. F. COOPER, B.A. (Cantab.), F.C.S. and W. A. B. WALLING.

(*From the Cooper Laboratory for Economic Research, Watford.*)

Introduction.

The experiments described in the present communication were made with the object of determining the insecticidal effect of various chemicals, a large number of which, hitherto, have never been actually employed as insecticides[1]. Most of these chemicals are already articles of commerce, whilst the remainder, if they should prove to be effective, could be produced on a commercial scale, if the demand arose.

The selection of the chemicals used was purely haphazard, the immediate object being to eliminate the least promising, and to gain such insight as would lead to a later and more precise series of experiments with the more promising compounds; also the range of selection was as wide as possible, and this has been justified by the results, as some of the most efficient compounds were unusual ones.

The choice of a suitable pest on which to work was also a matter for some consideration, especially as it was desired that the preliminary investigation should have as high a practical value as possible.

Some time ago, the authors were investigating the effects of different chemical reagents upon the eggs of Lepidoptera, but, for various reasons, they were forced to the conclusion that *ova* are unsuitable subjects for experiments of this nature, and the work was abandoned. These objections do not apply to the *larva*, and the question resolved itself into the choice of a form which was convenient to handle, and obtainable in quantity through the greater part of the year. Muscid larvae conform to these conditions, and as insecticidal methods of dealing with these, particularly in connection with the maggot-fly pest of sheep, and the dissemination of disease by house-flies, have been the subjects of much attention in recent years, one or other of these appeared to be eminently suitable.

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[1] Since this paper was written, the almost universal state of war has created conditions which are unprecedented in their possibilities for the propagation of disease by flies. In those areas which are at present the scene of military operations, the problem of dealing with flies, as agents in the transmission of disease, is likely to become acute in the immediate future; and we venture to hope that our results, incomplete as they are, may possibly afford some useful suggestions to those who are undertaking an active campaign against this menace.

The problem of the Maggot-fly pest is one which specially appeals to us, and, as the subject of the relation of the House-fly to the transmission of disease has been adequately dealt with by others, it was decided to use the larval form of one of our English Maggot-flies as a subject for experiment. The larva of *Calliphora vomitoria*, the common Blue-bottle, is readily obtainable at all times from dealers in anglers' requisites and consequently was selected as the most convenient species.

We realise that the most satisfactory manner of attacking the Maggot-fly problem would be in the nature of field experiments on the actual species, but the preliminary sorting out of likely chemicals is carried out most conveniently in the laboratory, so that it is only necessary to experiment with a small selection when the opportunity for field experiments presents itself.

The most obvious means of protecting sheep against the ravages of the Blow-fly are (*a*) the application to the fleece of some substance repellent or distasteful to the flies; Lavender Oil has been recommended in this category: (*b*) the application to the fleece of some compound actually poisonous to the Blow-fly or its larva. Our experiments fall, therefore, under these two main divisions.

As is usual in this class of investigation, very variable and contradictory results have been encountered. These are due to unknown or uncontrollable conditions, and such sources of error can only be eliminated by repetition of the experiment under slightly varying conditions.

The appearance of a Chalcid fly, which attacked the larvae in our later experiments, and, it is to be feared, seriously vitiated the results, unfortunately prevented us from confirming many of the results obtained, and this must be our excuse for publishing results obviously incomplete, and, in many respects, unsatisfactory. It is hoped, nevertheless, that the results obtained will serve as some guide to further work, especially in the direction of field experiments.

The actual manner in which the Blow-fly larva is killed by an insecticide, whether by absorption through the skin, ingestion, by suffocation due to the blocking of the spiracles, or by some other means, though a very important question, is outside of the province of this paper.

The Maggot-fly species, chiefly responsible for the damage to sheep in Australia, are *Calliphora oceaniae*, *C. villosa*, *C. rufifacies*, and *Lucilia caesar*. It is impracticable to employ any of these species for experimental work in this country, and, as the exact species is of little importance in preliminary work, for reasons specified above, the larva of the common "blue-bottle" (*Calliphora vomitoria*) was used.

At first, the larvae were all bred from flies captured locally. Large pieces of horse-flesh were exposed in a room, suitable precautions having been taken to prevent the escape of the flies, and a moderate supply of larvae was readily obtained. Owing, however, to the large number of chemicals for trial, this supply of maggots was inadequate, and larger supplies had to be obtained from a dealer in angling requisites. Buckets of cow manure had been placed in the breeding room, as it was thought that this might hasten the development of the flies, and, as prior to the introduction of purchased maggots and the cow manure, healthy larvae were always obtained, we suspect one or other of these as the source of the *chalcid* infection which upset our experiments. The mortality in our controls so long as we were using home-reared larvae was usually very low, and rarely exceeded 10 %. In the later experiments, however, the mortality in the controls increased to an excessive degree, and in not a few cases exceeded that of the chemically treated larvae! The *chalcid* infestation increased to such a degree, that very few of the pupae developed, and the investigation had to be abandoned[1].

A. *Experiments with Compounds presumably deterrent to the Adult.*

For this purpose, slabs of horse-flesh, of about 1 lb. in weight, were placed in shallow cardboard boxes, and the exposed surface dusted over with the reagent, suitably diluted with precipitated chalk. The boxes were then exposed to the flies. They were examined daily, and careful note taken of any "blowing." Controls, of untreated meat, were similarly exposed.

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[1] The species of *Chalcid* fly which caused the trouble is unknown. Specimens were sent to the British Museum for the purpose of determination, but were reported as unknown.

The astounding mortality caused by this infection suggests a means of controlling the Blow-fly pest in pastoral countries, and we have since heard that Mr Froggatt, Chief Entomologist to the New South Wales Government, is carrying out experiments with this end in view.

The following table gives the results of this experiment.

TABLE I.

The chemical was diluted with precipitated chalk, dusted on to the slabs of horse-flesh, which were then exposed to the Blow-flies for a period of 17 *days.* + *signifies blown.*

Substance	% of reagent in powder	Result	No. of days before flesh was blown
Zinc Oxide	10·0	+	7
Copper Carbonate	10·0	+	13
Sulphur	10·0	+	17
Arsenic Sulphide	10·0	+	11
Nitrobenzene	3·3	+	11
Eucalyptus	2·2	+	11
Clove Oil	1·7	+	11
Turpentine	1·6	+	7
Amyl Acetate	2·0	+	7
Methyl Salicylate	1·5	—	—
Cedarwood Oil	1·6	+	7
Oil of Camphor	2·0	+	13
Pyridene	2·1	+	7
p-Nitraniline	3·3	—	—
Aniline	1·7	+	7
p-Nitrophenol	3·3	+	7
Trichlorphenol	3·3	+	7
o-Nitrophenol	3·3	+	7
β-Naphthylamine	3·3	+	7
β-Naphthol	3·3	+	17
Oxalic Acid	10·0	+	11
Borax	10·0	+	17
Picric Acid (moist)	1·0	—	—
Creosote	4·4	—	—
Green Oil	4·1	—	—
Boracic Acid	10·0	—	—
Fusel Oil	1·15	—	—
Pine Oil	1·15	—	—
Alizarine Oil	1·2	—	—
Origanum Oil (brown)	1·0	—	—
Mustard Oil	1·0	—	—
Sod Oil	1·3	—	—
Lavender Oil	0·16	+	6
Aniseed Oil	0·33	+	6
Ginger	1·0	+	6
Iodoform	0·7	—	—
Dimethylaniline	2·2	—	—
Quinoline	1·5	—	—
Butyric Acid	1·8	+	6
Saxin	0·07	+	6
Allyl Alcohol	1·0	—	—
Alion	1·6	—	—
Saponin	10·0	—	—

Controls all badly blown within seven days.

As a result of this series of experiments, it is evident that a very considerable number of chemicals are deterrent to the flies and are capable of protecting horse-flesh from their ravages. Later in the summer, when the flies were much more numerous, this series of experiments was repeated with the same powders. In this case, all controls became blown in one day, and the protective effect of the powders was not nearly so marked as in the first series. Most of the pieces of flesh became blown in one to four days, immunity being only conferred by the powders containing the following chemicals: copper carbonate, nitrobenzene, borax, picric acid (calcium carbonate), creosote, sinapis oil, and aniseed oil.

The exact means by which this immunity is conferred is unknown. Clearly the sense organs of the fly itself are affected. Assuming the fly selects a spot for egg-laying from the smell, *e.g.* of decaying meat, *i.e.* to be guided by chemotaxis affecting "smell organs," these compounds influenced the smell of the horse-flesh by the flies. The deterrent compounds may have acted solely as preservatives and so prevented the decay of the horse-flesh that the flies failed to recognise it. On the other hand, either the taste or the smell of the compounds themselves may have been obnoxious to the flies.

It may be pointed out that the experiments in this section deal with chemicals obnoxious to the *adult fly*. The following sections have to do with chemicals obnoxious or toxic to the *larvae*. The results therefore are not comparable.

B. *Experiments with Compounds, presumably toxic to the larvae.*

The very large scale on which a successful insecticide would have to be applied precludes the use of a pure chemical and necessitates its dilution either by solution, emulsion in a suitable vehicle or admixture with an inert powder. Obviously the vehicle must be cheap and non-injurious to the sheep or its fleece. The following suggest themselves as being suitable in this respect: water, or paraffin as liquid vehicles, and some such material as precipitated chalk for powders. Many chemicals soluble in an oil but insoluble in water might be applied in the form of an emulsion. On account of its property of "creeping," paraffin might prove valuable. It is a solvent of many chemicals and its price would not be prohibitive, especially if it were applied in a fine spray or as an emulsion.

Precipitated chalk also appears to be quite practicable and has important properties for the purpose. It is extremely fine, and experiments carried out in the laboratory suggest that it could be blown into the wool as a spray. Oils are readily absorbed by precipitated chalk, consequently this is a very useful method of diluting them. Non-acid solids in powder form can be mixed with it. Chalk is quite neutral and in no way affects the wool. It would not, like lime, combine with soap, so that the treated wools would scour well and dye evenly. Precipitated chalk is obtained in large quantities as a waste product in the process of water softening and costs little more than freightage.

In Australia, water is not altogether desirable as a vehicle for treating sheep; it is not always very plentiful and it sometimes causes damage to the wool, which is usually long just at the time when it is necessary to apply the remedy. There is also the difficulty in wetting the fleece with an aqueous solution, but this is overcome to a considerable extent by the addition of an emulsion.

The use of an active chemical dissolved in an inert oil which is then emulsified by the addition of soap or some other emulsifying agent has certain advantages. It affords a means of applying chemicals which are insoluble in water. The emulsified liquid would also possess a high wetting power, a most desirable property where a greasy fleece is concerned.

Many substances are efficient insecticides in the form of vapour, and as the larvae breathe through spiracles, the method appeared to be worthy of trial. The treatment of lung-worm infection in sheep by inhalation of suitable vapours has been practised with some success in S. America and it would appear that some modification of this process might also be applied to the treatment of the fly pest, though the numbers of sheep to be treated might render it impracticable. A few experiments were carried out with the object of observing the effect of various vapours on larvae.

In determining the susceptibility of the larvae to various chemicals, therefore, our experiments fall under three heads:

(*a*) Those with a powder basis.
(*b*) Those with emulsions.
(*c*) Those with vapours.

It is obviously impossible in this country to carry out experiments on living sheep with the very large number of chemicals involved. It was, however, desirable that our laboratory experiments should simulate natural conditions so far as possible, and, for this reason, our earlier experiments were carried out on pieces of fresh sheep-skin. The skin was cut into rectangular pieces 12 × 8 inches and these were nailed on to a large board, wool uppermost, strips of wood being nailed between the different pieces, to prevent the larvae from migrating from one to another. The chemical to be tested was mixed with a definite proportion of precipitated chalk, a piece of hide dusted with the medicated powder and a definite number of larvae placed upon it. The hide was then covered with a piece of muslin, which was tacked down all round to the wooden partition strip, and the whole then placed aside for the larvae to develop. Untreated pieces of skin were also set aside to act as controls. The results are given in Table II.

TABLE II.

A definite number of larvae were placed on a piece of sheep-hide, the wool side of which had been previously treated with the active substance diluted with precipitated chalk.

Substance	% reagent in powder	% of larvae not pupating
1. Zinc Oxide	10·0	68
2. Copper Carbonate	10·0	14
3. Sulphur	10·0	0
4. Arsenic Sulphide	10·0	66
5. Nitrobenzene	·03	38
6. Eucalyptus	·02	20
CONTROL for expts. 1–3	—	12
CONTROL for expts. 4–6	—	6

The method was not successful; the pieces of skin dried up and often putrefied. Further, the larvae ate their way through the skin and crawling beneath it, out of the reach of the powders, pupated there in a manner quite impossible under natural conditions. Considerable difficulty was experienced in obtaining supplies of fresh skin suitable for the purpose. The length of the fleece varied very greatly on different skins, this, of course, introducing another undesirable factor. It was evident that, for comparative results, this method was useless and that some substitute for sheep-skin must be found.

Though differing greatly from wool, sand and sawdust appeared to be most convenient for the purpose. Definite quantities of these can be employed, and they are readily mixed with the substance under investigation. It was thought advantageous to make experiments both with sand and sawdust, as they differ considerably in one very important point, which might—and evidently did—have some effect on the results;

namely, the power of taking up or "adsorbing" substances. This property is possessed by sawdust but not by ordinary coarse sand. Wool has this property of adsorbing substances to a marked degree: further, this property is selective, inasmuch as wool adsorbs basic compounds more readily than acidic ones, the dyeing of wool being based upon this property. For this reason many basic substances are included in our list of possible toxic agents as pyridine, aniline, nicotine, β-naphthylamine, *p*-nitraniline.

(*a*) *Powder experiments.*

A definite number of larvae were shaken with the powder so as to be well covered with it, then carefully transferred to a glass jar containing sand or sawdust. The jar was covered with muslin and the number of flies which developed noted. The results are given in Table III. The powder consisted of the toxic agent diluted with dry precipitated chalk; the percentage of the toxic agent in the powder is given in column 2.

TABLE III.

Showing the different effect of sawdust and sand basis.
Larvae shaken in powder and then placed in sawdust or sand.

Powder	% reagent in powder	Sawdust basis Mortality %		Sand basis Mortality %	
		Expt.	Control	Expt.	Control
Zinc Oxide	10·0	28	8	32	36
Copper Carbonate	10·0	24	8	36	36
Sulphur	10·0	4	8	24	36
Arsenic Sulphide	**10·0**	**88**	**8**	**68**	**36**
Nitrobenzene	3·3	16	8	28	36
Eucalyptus	2·2	28	8	20	36
Oil of Cloves	1·7	4	8	52	36
Turpentine	1·6	8	8	12	12
Amyl Acetate	2·0	4	8	32	12
Methyl Salicylate	1·5	4	8	8	12
Cedarwood Oil	1·6	8	4	12	12
Oil of Camphor	2·0	20	4	12	12
Pyridine	2·1	8	4	24	8
p-Nitraniline	3·3	16	4	20	8
Aniline	1·7	4	4	4	8
p-Nitrophenol	3·3	12	4	8	8
Trichlorphenol	3·3	8	8	12	8
o-Nitrophenol	3·3	20	8	16	8
β-Naphthylamine	3·3	8	8	0	16
β-Naphthol	3·3	0	8	4	16

TABLE III (*continued*).

Powder	% reagent in powder	Sawdust basis Mortality % Expt.	Control	Sand basis Mortality % Expt.	Control
Oxalic Acid	10·0	12	8	0	16
Borax	10·0	12	8	8	16
Picric Acid (moist)	1·0	8	4	4	16
Creosote	4·4	0	4	32	16
Green Oil	4·1	4	4	12	0
Boracic Acid	10·0	8	4	52	0
Fusel Oil	1·15	4	4	36	0
Pine Oil	1·15	24	4	0	0
Alizarine Oil	1·2	4	4	12	0
Origanum Oil (brown)	1·0	4	4	0	0
Sinapis Oil	1·0	8	4	12	8
Sod Oil	1·3	8	4	24	8
Oil of Lavender	0·16	12	4	12	8
Aniseed Oil	0·33	8	4	20	8
Ginger	1·0	16	8	24	8
Iodoform	0·7	12	8	12	8
Dimethylaniline	2·2	16	8	4	8
Quinoline	1·5	8	8	8	8
Butyric Acid	1·8	0	12	20	8
Saxin (in alc.)	0·07	4	12	8	8
Allyl Alcohol	1·0	64	12	0	8
Aloin	1·6	4	12	8	8
Saponin	10·0	4	12	—	—

The results obtained in this series of experiments are extremely erratic; this might conceivably be chiefly due to two causes.

(*a*) Death might be due to starvation; the high mortality in the controls pointed to this;

(*b*) Larvae of mixed ages were employed, and these might have very different powers of resistance towards the toxic agent.

The next series of experiments was devised to test these two points. The young and old larvae were kept apart and experiments carried out on each. As before, a definite number were shaken with the powder and then placed in a glass jar in sand, meat being added in each case to prevent death by starvation.

The results are shown in Table IV.

TABLE IV.

Showing the different powers of resistance of old and young larvae. Larvae rolled in powder and then placed on sand.

Powder	% reagent in powder	Young larvae Mortality %		Old larvae Mortality %	
		Expt.	Control	Expt.	Control
Zinc Oxide	10·0	56	0	0	4
Copper Carbonate	10·0	16	0	0	4
Copper Carbonate	10·0	16	0	0	4
Sulphur	10·0	88	0	4	4
Arsenic Sulphide	**10·0**	**100**	**0**	16	4
Arsenic Sulphide	1·0	64	0	8	4
Nitrobenzene	**3·3**	**100**	**0**	16	4
Nitrobenzene	1·0	44	0	0	4
Eucalyptus	**2·2**	**100**	**0**	8	4
Eucalyptus	10·0	16	0	0	4
Oil of Cloves	1·7	20	0	12	4
Oil of Cloves	5·0	20	0	12	4
Turpentine	10·0	20	0	12	4
Methyl Salicylate	**10·0**	**100**	**32**	—	—
Cedarwood Oil	**1·6**	**100**	**32**	0	20
Oil of Camphor	2·0	44	32	—	—
Pyridene	5·0	20	32	4	20
Pyridene	10·0	12	32	12	20
***p*-Nitraniline**	**3·3**	**100**	**32**	4	20
o-Nitrophenol	3·3	84	28	8	4
β-Naphthylamine	**3·3**	**100**	**28**	40	4
β-Naphthol	10·0	20	28	4	4
Oxalic Acid	**10·0**	**100**	**28**	56	4
Borax	**10·0**	**100**	**28**	20	4
Picric Acid (moist)	**10·0**	36	28	**100**	**4**
Oil of Lavender	0·16	60	12	0	0
Oil of Lavender	1·0	84	12	68	40
Aniseed Oil	0·33	24	12	0	0
Aniseed Oil	1·0	0	12	0	0
Ginger	1·0	96	12	4	0
Ginger	5·0	0	12	0	4
Dimethylaniline	**2·2**	40	16	**100**	**4**
Quinoline	**1·5**	**100**	**16**	20	4
Allyl Alcohol	1·0	96	16	0	4
Allyl Alcohol	0·5	20	16	0	4
Aloin	1·6	20	16	0	4

The results are still somewhat erratic, but it is evident that the young larvae are far more susceptible to the influence of the poisons than are the old larvae. This shows that it is undesirable to carry out experiments with larvae of mixed ages, and suggests that in preventive measures against the blow-fly pest it is necessary that the remedy should be applied before the larvae hatch, or in the very earliest days of the larval stage.

The higher mortality of the young larvae may possibly be explained as follows. Young blow-fly larvae secrete a fluid which digests tissue. The resulting liquid is then re-absorbed. The toxic effect of a toxic compound, *e.g.* arsenic sulphide, on young larvae may therefore be conceivably due to an interference with their digestive faculties, and, in this case, the compound would be acting as a stomach poison, without, however, entering the larva. The results with young larva would thus be comparable to those obtained with caterpillars, when *e.g.* their digestive organs had been affected. Old larvae, ceasing to feed, no longer secrete digestive fluid, so that the effect of the compound on these would be confined to that of a contact poison, and would chiefly act upon the respiratory organs.

Table V gives the results of further experiments which only differ from those recorded in Table IV by the fact that the powder under investigation was mixed with sufficient sand to reduce the proportion of toxic agent to one-tenth of the original strength. A definite number of larvae were placed in the mixture of sand and powder. As before, the mixture was kept in glass jars covered with muslin. The series contains experiments on young, and old, larvae.

The experiments on the old larvae were for the most part vitiated by the Chalcid infection, to which reference has already been made. A great mortality, sometimes amounting to 100 %, was observed in the control experiments and can only be explained by this fact. Only those experiments in which the controls are not apparently affected appreciably by the parasitic fly are therefore recorded.

The higher susceptibility of the young larvae is most marked and, considering the small percentage of toxic substance present, many of the substances used gave results which lead us to believe that they are worthy of a practical trial in the field. Arsenic sulphide, nitrobenzene, methyl salicylate, cedarwood oil, *p*-nitraniline, borax, picric acid, dimethylaniline, quinoline, as in the previous series, have all given highly satisfactory results; whilst, in addition, copper carbonate, oil of cloves, turpentine, β-naphthol, creosote, green oil, boracic acid, fusel oil, sinapis and aniseed oil, all seem to have a poisonous effect on the young larvae.

TABLE V.

Powder mixed with sand and larvae placed in it.

Powder	% reagent in sand basis	Young larvae Mortality %		Old larvae Mortality %	
		Expt.	Control	Expt.	Control
Zinc Oxide	1·0	68	0	—	—
Copper Carbonate	1·0	84	0	—	—
Copper Carbonate	**1·0**	**100**	**0**	0	0
Sulphur	1·0	36	0	—	—
Arsenic Sulphide	**1·0**	**100**	**0**	—	—
Arsenic Sulphide	**0·1**	**100**	**0**	68	0
Nitrobenzene	**0·33**	**100**	**0**	—	—
Nitrobenzene	**0·1**	**100**	**0**	**80**	**0**
Eucalyptus	0·22	40	0	—	—
Eucalyptus	1·0	0	0	0	0
Oil of Cloves	0·17	76	0	—	—
Oil of Cloves	**0·5**	**100**	**0**	**76**	**0**
Turpentine	0·16	0	0	—	—
Turpentine	**1·0**	**100**	**0**	16	0
Amyl Acetate	0·17	52	0	—	—
Methyl Salicylate	**0·15**	**88**	**0**	—	—
Methyl Salicylate	1·0	—	—	16	0
Cedarwood Oil	**0·16**	**92**	**0**	—	—
Oil of Camphor	0·17	20	0	—	—
Pyridene	0·17	80	0	—	—
Pyridene	0·5	12	0	0	0
Pyridene	1·0	32	0	0	0
p-Nitraniline	0·33	40	0	—	—
***p*-Nitraniline**	**0·5**	**100**	**0**	0	0
Aniline	0·17	40	0	—	—
p-Nitrophenol	0·33	60	0	—	—
Trichlorphenol	0·33	52	0	—	—
o-Nitrophenol	0·33	96	0	—	—
β-Naphthylamine	0·33	48	0	—	—
β-Naphthol	0·33	48	0	—	—
β-Naphthol	**1·0**	**100**	**0**	20	0
Oxalic Acid	1·0	52	0	—	—
Borax	1·0	96	0	—	—
Picric Acid (moist)	0·1	88	0	—	—
Picric Acid (moist)	**1·0**	**100**	**8**	0	0
Creosote	**0·44**	**100**	**0**	—	—
Creosote	0·1	28	8	76	0
Creosote	**1·0**	**100**	**8**	**100**	**0**
Green Oil	**0·4**	**88**	**0**	—	—
Boracic Acid	**1·0**	**100**	**0**	—	—
Fusel Oil	**0·115**	**100**	**0**	—	—
Pine Oil	0·115	20	0	—	—
Pine Oil	**0·5**	**92**	**8**	32	0
Alizarine Oil	0·12	0	0	—	—
Origanum Oil	0·11	16	0	—	—
Sinapis Oil	**0·11**	**84**	**0**	—	—
Sinapis Oil	**0·5**	**100**	**0**	36	0
Sod Oil	0·13	64	0	—	—
Sod Oil	0·5	88	0	0	0
Oil of Lavender	0·016	60	0	—	—
Oil of Lavender	0·1	96	0	0	0
Aniseed Oil	0·033	36	0	—	—
Aniseed Oil	**0·1**	**100**	**0**	12	0
Ginger	0·1	—	—	0	0
Ginger	0·5	24	0	0	0
Iodoform	**0·07**	**100**	**40**	—	—
Dimethylaniline	**0·22**	**100**	**40**	—	—
Quinoline	**0·15**	**100**	**40**	—	—
Butyric Acid	0·18	52	40	—	—
Saxin (in alc.)	0·007	36	40	—	—
Allyl Alcohol	0·1	24	40	—	—
Allyl Alcohol	**0·05**	**80**	**8**	0	0
Aloin	0·16	76	40	—	—

TABLE VI.

Powder mixed with sawdust and larvae placed in it.

Powder	% reagent in sawdust basis	Young larvae Mortality % Expt.	Young larvae Mortality % Control	Old larvae Mortality % Expt.	Old larvae Mortality % Control
Zinc Oxide	1·0	32	46	0	36
Copper Carbonate	1·0	56	46	44	36
Copper Carbonate	1·0	40	46	4	36
Sulphur	1·0	80	46	60	36
Arsenic Sulphide	1·0	92	46	92	36
Arsenic Sulphide	0·1	8	46	12	36
Nitrobenzene	**0·33**	**100**	**46**	**92**	**36**
Nitrobenzene	0·1	20	46	12	36
Eucalyptus	0·22	72	46	44	36
Eucalyptus	1·0	0	46	8	36
Oil of Cloves	**0·17**	**80**	**46**	0	36
Oil of Cloves	0·5	8	46	16	36
Turpentine	0·16	36	46	16	36
Turpentine	1·0	16	46	12	36
Amyl Acetate	0·17	44	46	76	36
Methyl Salicylate	0·15	40	46	56	36
Cedarwood Oil	0·16	24	46	44	36
Oil of Camphor	0·17	64	46	52	36
Pyridene	0·17	64	46	64	36
Pyridene	0·5	28	46	4	36
Pyridene	1·0	0	46	16	36
p-Nitraniline	0·33	16	46	88	36
p-Nitraniline	0·5	16	46	0	36
Aniline	0·17	16	46	60	36
p-Nitrophenol	0·33	68	46	48	36
Trichlorphenol	0·33	52	46	48	36
o-Nitrophenol	0·33	28	46	84	36
β-Naphthylamine	0·33	40	46	96	36
Naphthol	0·33	0	46	0	36
Naphthol	1·0	0	46	16	36
Oxalic Acid	1·0	28	46	76	36
Borax	1·0	28	46	20	36
Picric Acid (moist)	0·1	36	46	20	36
Picric Acid (moist)	1·0	12	46	12	36
Creosote	0·44	80	46	100	36
Creosote	0·1	16	46	60	36
Creosote	1·0	36	46	8	36
Green Oil	0·4	44	46	40	36
Boracic Acid	1·0	40	46	44	36
Fusel Oil	0·115	28	46	40	36
Pine Oil	0·115	40	46	24	36
Pine Oil	0·5	20	46	0	36
Alizarine Oil	**0·12**	**92**	**46**	20	36
Origanum Oil (brown)	0·11	70	46	60	36
Sinapis Oil	0·11	16	46	0	36
Sinapis Oil	0·5	56	46	0	36
Sod Oil	0·13	36	46	0	36
Sod Oil	0·5	16	46	28	36
Oil of Lavender	0·16	20	46	40	36
Oil of Lavender	0·1	12	46	4	36
Aniseed Oil	0·033	20	46	16	36
Aniseed Oil	0·1	8	46	4	36
Ginger	0·1	24	46	52	36
Ginger	0·5	24	46	0	36
Dimethylaniline	0·22	76	46	**100**	**36**
Quinoline	0·15	52	46	52	36
Butyric Acid	0·18	16	46	56	36
Saxin (in alc.)	0·007	28	46	40	36
Allyl Alcohol	0·1	40	46	8	36
Allyl Alcohol	0·05	32	46	4	36
Aloin	0·16	36	46	20	36

Table VI is a summary of the results of a further series of experiments which were a duplicate of those recorded in Table V, except that sawdust was substituted for sand.

The figures given for the controls in the experiments on the old and young larvae represent the average mortality in *seven* control experiments, each on 25 larvae. There was, however, a very marked variation in the mortality of the controls themselves, and, as this had not been observed to any great degree before the advent of the Chalcid fly, is to be attributed to the ravages of the latter. The very frequent discrepancies, obtained in this series, in which the mortality in the experiment is markedly lower than in the control, may also in many cases be caused by the Chalcid fly. Comparing the results on *sawdust* with those on *sand*, it is at once evident that the toxic agents are not nearly so effective in the case of the former. This is merely a confirmation of a result obtained in an earlier series of experiments, but it is of importance as indicating that the high toxic values, which various substances show with sand, would in all probability be reduced in actual practice, owing to the relatively higher adsorptive powers of the fleece.

The highly poisonous nature of arsenic sulphide, nitrobenzene and creosote is again confirmed in this series of experiment.

(*b*) *Experiments with emulsions.*

The experiments with emulsions include most of the compounds which previous series of experiments have shown to be fairly efficient, together with some new preparations.

The actual experiments were carried out as follows:

40 gms. of sawdust were taken and sprayed with an emulsion containing 1 % of the active constituent. Sufficient liquid was used to make the sawdust just damp, the sawdust being well mixed during the spraying. Fifty larvae were then placed in the sawdust, in a glass jar, the mouth of which was covered with a piece of muslin. The results are given in Table VII.

Satisfactory results have only been given by two of the preparations, namely those containing safrol, and β-naphthol and sulphur. The general results are not nearly so good as those given by the powders and the series was therefore not extended further, except that some of the same poisons were tried at a higher concentration. The whole of these experiments were, however, so badly infested with the Chalcid fly that the results were useless.

TABLE VII.

Emulsions.

	Mortality %	
Reagent	Expt.	Control
Creosote	16	12
Turpentine	8	12
Methyl Salicylate	24	12
Crude Pyridine	30	12
Safrol	**92**	**12**
Indol	16	0
Preparation containing Green Oil and Pyridine	50	8
Preparation containing Sulphides of carbon and potash	0	8
Preparation containing β-Naphthol	2	8
Preparation containing Green Oil	20	8
Preparation containing Resin Oil	18	8
Preparation containing β-Naphthol and Potassium Sulphide	4	8
Preparation containing β-**Naphthol and Sulphur**	**100**	**8**
Preparation containing Potassium Sulphide	14	8

(c) *Vapour experiments.*

In these experiments a large number of larvae were placed in a wide glass tube, about 1½ ins. in diameter and 9 ins. long, one end of which was attached to an aspirator and the other to a "U" tube containing the material to be tested; so that the air which was drawn over the larvae by the aspirator became saturated with vapour in the "U" tube. The larvae were subjected to this treatment for 3½ hours; then they were placed in sawdust in jars.

The controls were placed in tubes through which pure air was drawn in the same manner.

TABLE VIII.

Vapour Experiments.

Reagent	Mortality %		Duration of exposure to vapour		Remarks
	Expt.	Control	h.	m.	
Benzene	22	10	4	30	
Xylol	36	10	4	30	
Aniline	6	10	5	—	
Acetone	62	10	5	—	
Nicotine	10	16	3	30	
Nitrobenzene	42	16	3	30	
Clove Oil	12	16	3	30	
Eucalyptus	8	16	3	30	
Turpentine	16	14	3	30	
Amyl Acetate	12	14	3	30	
Sinapis Oil	8	14	3	30	Oil frothed freely.
Cedarwood Oil	18	14	3	30	
Fusel Oil	8	14	3	30	
Pine Oil	2	14	3	30	
Origanum Oil (brown)	12	14	3	30	
Creosote	10	14	3	30	
Green Oil	16	14	3	30	
Pyridine	**100**	**14**	3	30	
Dimethylaniline	28	6	3	30	
Carbon Bisulphide	8	6	3	30	
Methyl Salicylate	18	6	3	30	
Butyric Acid	14	6	3	30	
Mono-Brombenzene	**94**	**0**	3	30	
Amyl Alcohol	0	0	3	30	
Acetic Acid	0	0	3	30	
Ethylene Bromide	0	0	3	30	Larvae inert when removed.
Chloral Hydrate	**100**	**0**	3	30	Larvae most inert on removal.
Iodoform	6	0	3	30	
Aniseed Oil	0	0	3	30	
Ethyl Acetate	**100**	**0**	3	30	Larvae all inert on removal.
Iodine	**78**	**0**	—		
Alcohol Absolute	18	0	—		
Ammonia	10	0	—		
Acetone	8	0	—		
Bone Oil	0	38	—		
Carbon Tetra Chloride	68	38	—		
Ammonium Sulphide	0	38	—		
Aldehyde	0	38	—		

The following seemed to be most effective:

Mono-Brombenzene, chloral hydrate, pyridine, ethyl acetate and iodine.

SUMMARY.

In conclusion, the preliminary character of our experiments may again be emphasised. Final conclusions as to the insecticidal value of any preparation can never be drawn, at least with any degree of satisfaction, from laboratory experiments alone. These should obviously be followed by field work under natural conditions, which unfortunately is not possible in this country.

The general results, which we summarise below, are intended therefore to afford some indication as to possible lines upon which future field work might profitably be pursued, rather than as definite recommendations of substances, by the employment of which the Blow-fly Pest may be controlled.

Of substances repellent to the Blow-fly, and therefore capable of protecting sheep from their ravages, the following appear to be the most suitable: methyl salicylate, *p*-nitraniline, picric acid, creosote, green oil, boracic acid, fusel oil, pine oil, alizarine oil, origanum oil, mustard oil, sod oil, iodoform, dimethylaniline, quinoline, allyl alcohol, aloin, saponin, copper carbonate, nitrobenzene, sinapis oil and aniseed oil.

For the application of toxic agents, a powder form has been found to be very convenient and efficient, precipitated chalk forming a suitable and cheap basis. The substances, applied in this form, which appear to be most toxic to the Blow-fly larva, comprise the following: arsenic sulphide, nitrobenzene, eucalyptus oil, methyl salicylate, cedarwood oil, *p*-nitraniline, *β*-naphthylamine, oxalic acid, borax, quinoline, allyl alcohol, picric acid, dimethylaniline, copper carbonate, oil of cloves, turpentine, *β*-naphthol, creosote, fusel oil, sinapis oil, aniseed oil and iodoform. Since the young larvae are much more susceptible than the old, in field work, the medicated powder should be applied either previous to, or in the very earliest days of, the larval stage.

Various vapours have been shown to be toxic to the Blow-fly larvae, and of these the most successful are brombenzene, chloral hydrate, ethyl acetate, iodine and pyridine.

ON DISEASES OF PLUM TREES CAUSED BY SOME SPECIES OF *CYTOSPORA*.

By W. N. C. BELGRAVE, B.A.

(*Research Scholar, University College, Nottingham, and formerly Exhibitioner, St John's College, Cambridge.*)

INTRODUCTION.

WITHIN the last few years, an increasing number of plum trees in the fruit plantations of Cambridgeshire have exhibited symptoms of a "die-back" disease, followed by the formation of pycnidia, from which red semi-gelatinous tendrils are exuded, these spore-masses belonging to the genus *Cytospora*, the members of which are recognised as conidial stages of various species of *Valsa*.

This investigation was commenced at Cambridge, at the suggestion of Mr F. T. Brooks, to whom I am much indebted for assistance. The work was continued at Nottingham, and I desire to express my thanks to Prof. J. W. Carr for permission to work in his laboratory.

No serious damage to mature plum trees, caused by members of the genus *Valsa*, has hitherto been recorded in England, though Massee (4) has described *Eutypella prunastri* (Sacc.) as attacking young plum stocks. Aderhold (1) in Germany, and Wormald (5) in this country, have described a strikingly similar disease of cherry trees caused by *Valsa* (*Cytospora*) *leucostoma*. As all efforts to obtain mature asci have failed, it is impossible to give the species of the fungus or fungi investigated with any approach to certainty.

FIELD OBSERVATIONS AND DESCRIPTION OF FRUCTIFICATIONS.

The diseased trees examined belonged to three varieties, Victoria, Prince of Wales, and Pond's Seedling. The trees attacked range from those 4–5 years old, up to the very largest, and the attack usually proves fatal. The first sign of attack is a withering of the leaves, usually progressing from the top of the tree downwards. Next, areas of bark, which may be on the main stem or a side branch, collapse and turn brown; on these, after a considerable interval, numerous crater-like or lenticular fructifications develop, which almost invariably prove to be pycnidia. A certain amount of gum appears at the junction areas, but considering the proneness of plum trees to gum production its presence cannot be taken as a characteristic of these diseases.

This general description holds for all the trees examined, but there are differences in detail considerable enough to warrant separate treatment. The following record indicates the nature of these.

A. *Victoria.*

All the specimens, with one exception, were from trees on plantations at Willingham, Cambridgeshire.

(1) A small shoot from an old tree, of which large lateral branches, six years of age, were affected. The shoot was dead. Numerous small crater-like swellings, 1 mm. in diameter, of the bark were in evidence, due to hard pycnidia, seated in the deeper cortical layers. These pycnidia were seated on a poorly developed, dirty-white stroma, sharply limited from the surrounding tissues by a dark tough "skin." Each pycnidium had a single central pore, and was many-chambered; the walls were lined with sparingly-branched hyaline conidiophores, so closely packed as to form a palisade-like tissue.

The spores, borne singly at the tips of conidiophore branches, were continuous, sickle-shaped, hyaline bodies, averaging 7 μ long, and 1·5 μ broad, with two oil bodies. In moist weather, they are extruded in enormous numbers as pink tendrils, semi-gelatinous at first, becoming horny on drying. On wetting, the tendrils disintegrate into their component spores.

(2) A tree about five years old, from the University Farm, Cambridge, was cut down in August, 1913, and kept exposed on the laboratory roof till the following October. It had then developed numerous pycnidia borne several on each erumpent oval dark stroma. As no traces of this fungus were present in August it must here have developed as a saprophyte. Each pycnidium had several pores. Pink tendrils were extruded, made up of spores similar to those described above, but 5 μ long by 1 μ broad.

(3) A tree nine years old, dead. This specimen was taken 18 ins. from the ground line region, which was also the point of attack. The upper part of the tree was still green. The stromata were black, erumpent, and lenticular; each bearing several pycnidia (Figs. 1 and 2). Over the exterior of each fissure was a thin white covering, specially

noticeable on wetting the bark. Dark red tendrils were extruded, spores as above, 5 μ by 1 μ.

(4) A piece of bark only. The stromata were large and pulverulent, nearly 5 mm. long, dark olive-green in colour, and sunken in the bark (Fig. 3). There was a sharp line of demarcation from the surrounding tissue. Each stroma bore several immature perithecia.

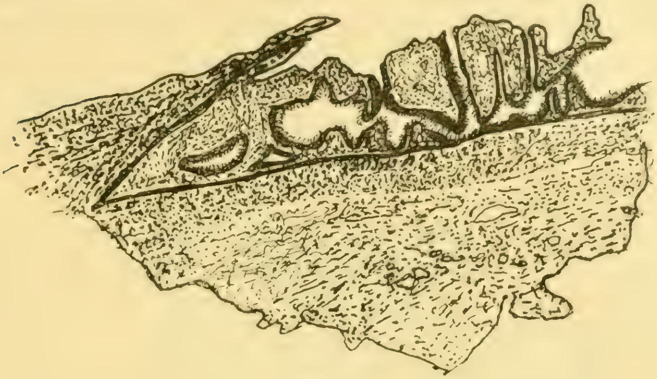

Fig. 2.

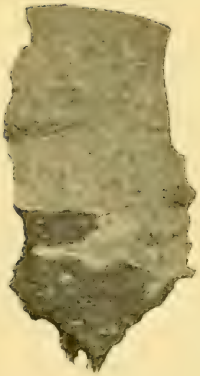

Fig. 1.

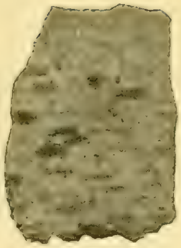

Fig. 3.

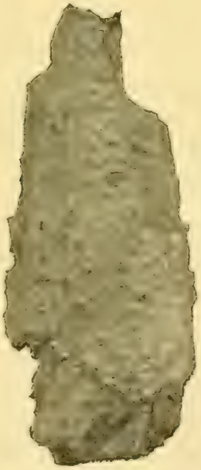

Fig. 4.

B. Prince of Wales.

(5) One mature tree was dead, two or three others were dying back from the top. All diseased trees were found at Histon, Cambridgeshire. Small, blue-green, sunken pycnidia were borne singly on poorly-developed stromata, erumpent on keeping (Fig. 4). Pink tendrils of the usual type were developed in a moist chamber.

C. Pond's Seedling.

(6) A mature tree at Long Sutton near Wisbech, one side of which was dying. The disease had spread from above downwards. Pycnidia were borne in groups on sunken, whitish, poorly-developed stromata. There were pink tendrils of spores, the dimensions of which were 5 μ by 1 μ. At the time Mr Brooks obtained this material the foliage of the parts of the tree affected was wilted and brown, presenting a scorched appearance.

(7) Piece of branch about 4 ins. in diameter from a tree at Long Sutton. Pycnidia and spores as in (6) above, but stromata erumpent, and dark-coloured.

It is possible broadly to divide the above into:

(*a*) Those with stromata well developed, dark coloured, and erumpent—Victoria (2) and (3) and Pond's Seedling (7).

(*b*) Those with stromata poorly developed, light coloured, and sunken—Victoria (1) and Pond's Seedling (6).

Too much stress, however, cannot be placed on this classification, in view of the great similarity of the spores and tendrils (except in the case of Victoria (3) where the dark-red tendrils and white crust point to a wider divergence) and also, as will be seen later, on account of the marked influence of media on pycnidial development.

The presence of a stroma more or less deeply seated in the bark, and sharply delimited from the surrounding tissues, indicates that all the fungi belong to the sub-genus *Leucostoma* of the genus *Valsa*, assuming, as is highly probable, that the stroma of the perithecial stage is similar to that bearing pycnidia. Beyond this it is not possible to go. The chief distinction from *Eutypella prunastri* appears to be in the production of well-marked tendrils of spores (cf. Massee, *l.c.*).

EFFECTS OF THE FUNGI ON THE TISSUES OF THE HOST.

The action of the fungi on the tissue of the host has been examined. The stains found most effective in this examination were Delafield's Haematoxylin, and the double stain Picric-Aniline Blue (picric acid being added to saturation).

It is found that the hyphae travel in the soft tissues of the bark, thence spreading laterally into the wood (cf. Fig. 5).

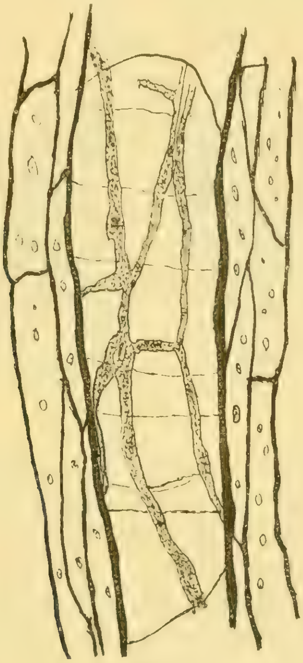

Fig. 5.

In all the tissues gum makes its appearance, in the bark and medullary rays as droplets; in the wood large masses are of frequent occurrence. As pointed out above, the diseased areas are easily identified by their collapsed bark. This collapse is caused by the death and decay of all living cells, only the fibrous cells remaining. It is usual to find above the junction areas of collapsed and healthy areas of bark, considerable lengths of red or brown wood, in which no hyphae can be traced, but gum is abundant (cf. Fig. 6). This phenomenon, however caused, is of common occurrence.

Lateral penetration of the hyphae takes place through the pits which abound in the tissues of the wood (cf. Fig. 7). This penetration is very slow, and often limited to the young wood, *e.g.* in a dead branch 8 ins. in diameter, from a Prince of Wales tree, hyphae could be found in the outermost $\frac{3}{4}$ ins., and in the inner part of this zone only in the medullary rays. In the vessels themselves the hyphae were limited to the outermost $\frac{1}{2}$ inch.

The cell walls are practically unaffected by these fungi, sections from a large dead branch failing to show any discoloration after prolonged treatment with Schultz's reagent; in consequence dead wood, although brittle, shows no signs of crumbling.

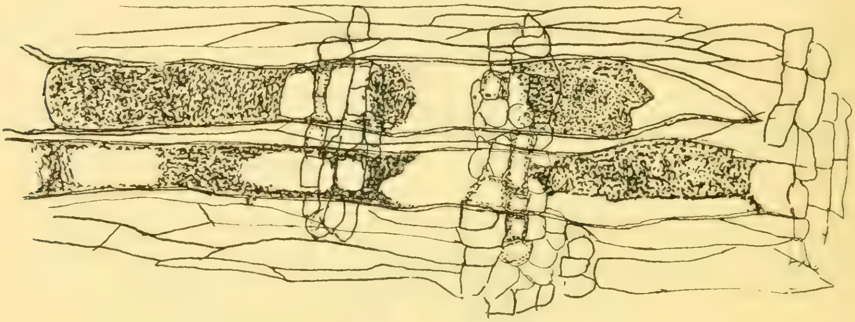

Fig. 6.

The formation of pycnidia is preceded by an aggregation of hyphae in the tissues of the bark to a small hard pustule, which gradually enlarges, splitting the bark in the process. Owing to the friability of diseased bark, this process could not be followed in detail.

In "Victoria (3)," the hyphae are dark coloured, large, fairly thick walled, and stain with difficulty; in the remainder, they are thin walled, narrow, hyaline, and stain readily. With this single exception, the above description applies to all the specimens examined.

CULTURE EXPERIMENTS AND CHARACTERS OF THE FUNGI IN PURE CULTURE.

All attempts to bring about maturation of the asci in the only perithecium found having failed, the description which follows applies entirely to cultures obtained from conidia.

Separate experiments in regard to the germination of spores were made with all the "strains" mentioned above, and there was complete agreement in the results attained.

Germination readily took place in twenty-four hours at room temperature (summer) in all natural nutriment media, *e.g.* grape juice, fruit extracts stiffened with agar or gelatine, and plum wood extract. Moistened strips of plum wood and solid media, such as carrots or potatoes, also gave good results.

No germination took place in distilled, rain, or tap water, or in water collected after slowly trickling down a healthy plum shoot.

The limits of vitality of the spores are not known, although (*a*) spores readily germinated after five days' soaking in water, when some nutrient material was added, (*b*) spores from tendrils which had been kept for four months in the dry laboratory air readily germinated, (*c*) spores from tendrils which had been kept three months in culture vessels in a saturated atmosphere, likewise germinated. These facts indicate considerable vitality within wide limits.

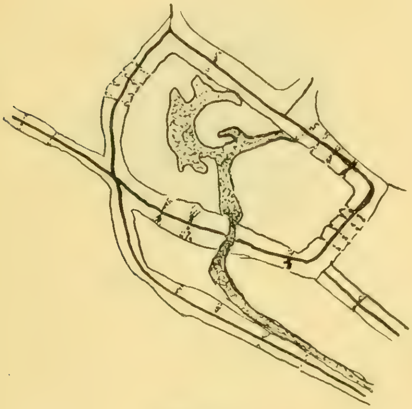

Fig. 7.

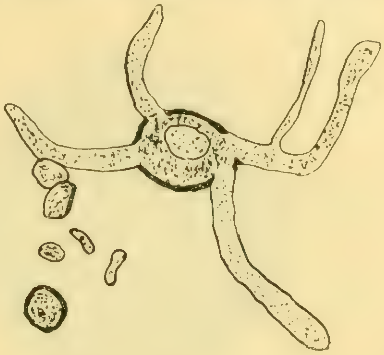

Fig. 8.

Germination tests were also carried out with artificial media. In those containing no nitrogen, such as solutions of glucose, saccharose, or either of these with the addition of phosphates, no germination took place. On the addition of nitrogen in simple combinations, such as ammonium salts, only a slight swelling resulted. Ammonium tartrate and non-poisonous nitrates give better results, and small germ-tubes were produced. The addition of organic nitrogen, *e.g.* peptone or albumen, brought about normal germination.

The details of germination are similar to those described by Aderhold (1). The sickle-shaped spores enlarge considerably, and in about ten hours, by swelling along the short diameter, are converted into spheres, from which eight to ten hours later 1–4 germ tubes protrude (cf. Fig. 8).

The isolation in pure culture of members of this genus is rendered easy by the production of tendrils of spores. A small piece of tendril is placed in a drop of sterile water, and spores transferred from this to the required medium. When large numbers of cultures were required it was found most convenient to make stock cultures on grape-gelatine, from which mycelial inoculations were carried out.

In nearly all cases, mycelial growth followed by pycnidial formation takes place with great rapidity.

There is an entire absence of any definite stroma, or limiting layer; the pycnidia being developed on small cushions of hyphae (cf. Fig. 9) and the hyphae of the general tissue passing gradually into conidiophores.

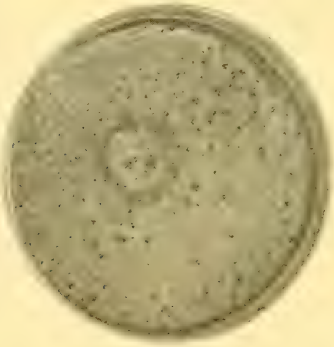
Fig. 9.

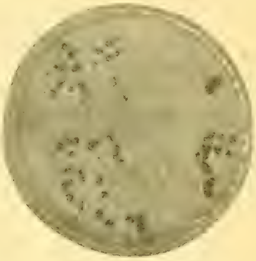
Fig. 10.

On cutting sections of a very young pustule (it is best to take one growing on agar material), it is seen to be a solid mass of interwoven hyphae. As growth continues, spaces appear, which later become chambers. These chambers are lined with sparingly-branched conidiophores, which are full of minute darkly staining granules, specially noticeable as the conidiophore walls stain only feebly. Later, conidia are abstricted from the tips of the conidiophores. One or more pores now appear in the pycnidia, through which drops of water are extruded, followed by the spores, either as pink tendrils, or more often, owing to the saturated atmosphere, as pink droplets.

The media employed included grape juice, raisin extract, plum wood extract, alone or stiffened with agar or gelatine; artificial nutrient solutions; and solids, *e.g.* potato, carrot, turnip, and plum wood.

Generally, it may be said that liquid media were not favourable to growth; gelatine cultures were marked by rapid and profuse mycelial growth at the expense of pycnidial formation, the pycnidia when formed being rudimentary; on agar, growth was slow, but pycnidia were abundantly formed.

Potato and turnip slices did not prove good media, growth was slow and pycnidial formation scanty. Carrot was highly satisfactory, large tendril-producing pycnidia being rapidly formed.

Growth on wood blocks, in the usual culture tubes, was very rapid on the surface, a dense mycelial felt being produced in five days. Penetration was however slow; in one case, no hyphae were at any depth greater than ¼ inch in a block inoculated two months before; after ten months penetration was complete. Pycnidia were formed in greater abundance on those blocks with bark attached; the bark is split by pressure from below, and through the fissures tufts of hyphae come to the surface; on these, pycnidia are developed in 3–4 weeks.

This formation of pycnidia on the surface of the medium, instead of being immersed, has been noted by Aderhold (1), and ascribed to the high moisture content of the air in culture vessels.

All spores produced in artificial culture were found to be of uniform size and shape, 5 μ by 1 μ, hyaline, continuous, and sickle-shaped, thus agreeing with those found in nature.

The following is a detailed description of certain peculiarities in some of the "strains"; for convenience each strain is described by the name and number of its host.

(*a*) *On raisin or grape-gelatine media.*

Victoria (1). Medium rapidly coloured black, hyphae remaining hyaline.

Victoria (2). Mycelium brown, no discoloration in medium.

Victoria (3). Hyphae dark, no discoloration.

Prince of Wales (5). Mycelium confined to the upper surface of medium, forming a light-brown skin. No discoloration of medium.

Pond's Seedling (6). No discoloration of medium. At the edge of the dish a peculiar "efflorescence" of the medium takes place, due to the production of snow-white, feathery aerial hyphae; these sometimes appear in a dish which is drying up.

(*b*) *On raisin, grape juice, or plum agar media.*

Victoria (1). Medium blackened. Pycnidia black, 1–2 mm. diameter covered with a light-grey mycelial felt (cf. Fig. 9).

Victoria (3). No discoloration of medium. Hyphae hyaline at first, dark later. Pycnidia as in (1).

Prince of Wales (5). Colourless mycelium. No discoloration. Pycnidia black, covered with grey felt.

Pond's Seedling (6). White mycelium. No discoloration. Formation of aerial hyphae similar to those produced on gelatine. Large white pycnidia, covered with a greenish felt (Fig. 10).

(*c*) *On potato agar media.*

Victoria (1). Greenish-black coloration of medium. Large pycnidia.

(*d*) *On wheat flour agar media.*

Victoria (1). No discoloration. Cream-coloured, almost invisible, mycelium. Grey-coloured large pycnidia.

(*e*) *On acid and alkaline media.*

By titration with normal acid or alkali, using phenol phthalein as an indicator, a series of raisin agar tubes was obtained, containing various concentrations of acid or alkali. It was found that the degrees of acidity or alkalinity limiting the growth of these fungi were 10 per cent. normal HCl and 5 per cent. normal NaOH.

Victoria (1). 10 per cent. normal HCl. Slow growth. No discoloration of medium.

5 per cent. normal HCl, neutral, and 5 per cent. normal NaOH. Normal growth, with discoloration of medium.

Pond's Seedling (6) and *Prince of Wales* (5). Normal growth at all concentrations, within above limits.

Formation of perithecia.

Prolonged but fruitless attempts were made to induce perithecial formation in culture. These included growing the fungi on pure agar, agar with a high concentration of nutrient material, acid and alkaline media, and upon wood. Cultures were also kept (*a*) at 28° C. for three months, (*b*) frozen, (*c*) exposed throughout the winter on the laboratory roof, (*d*) in the dark; but with negative results.

The recent work of Shear (3) and Harper (2) has indicated the existence of "strains" within the same species of fungus which behave differently in culture media. It seems probable that the fungi here investigated were conidial bearing strains only. The constant cultural differences described above point to the existence of different strains.

Inoculation experiments.

These have, so far, yielded negative results. A T-shaped cut was made in the bark of a healthy plum shoot, and a small piece of mycelium introduced under the edges of the cut.

Inoculations made in September, 1913, were examined a year later but no signs of infection could be found.

In view of the negative results yielded by the infection experiments up to the time of writing conclusive proof that the "die-back" disease under investigation is caused by the fungi in question is lacking. There is however strong presumptive evidence that they are responsible. Their presence in the tissues of the host in each case examined, the general character of the development of their fructifications on the diseased bark, the identity with or close relationship to *Cytospora leucostoma*, a parasitic fungus known to cause a strikingly similar disease on cherry trees, of the fungus isolated from the diseased areas of the affected plum trees in most cases examined, and the failure to find in the diseased parts any other organism to which the disease could be attributed, are points which, taken collectively, suggest that the fungi in question have caused the trouble. The lack of confirmative evidence from the infection experiments, although rendering absolute proof of the cause of the disease at this stage impossible, does not necessarily conflict with the view that these fungi are the cause, since it is always possible that the conditions necessary for successful infection did not obtain in the experiments already conducted.

SUMMARY.

(1) A disease or diseases of plum trees believed to be caused by one or more species of *Cytospora* has been described.

(2) The fungus isolated in most cases is closely related to or identical with *Cytospora leucostoma.*

(3) Complete germination of the spores took place only in presence of organic nitrogen.

(4) Pycnidia and spores were obtained in artificial culture, similar to those occurring in nature.

(5) Attempts to induce perithecial formation failed.

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EXPLANATION OF FIGURES.

Fig. 1. A piece of diseased bark of Victoria (3). Nat. size.
Fig. 2. T.S. of pycnidia of Victoria (3). ×80.
Fig. 3. A piece of diseased bark of Victoria (4). Nat. size.
Fig. 4. A piece of diseased bark of Prince of Wales (5). Nat. size.
Fig. 5. L.S. of diseased wood of Prince of Wales. ×300.
Fig. 6. L.S. of wood of diseased Prince of Wales branch, above hyphae. ×220.
Fig. 7. T.S. of diseased wood of Prince of Wales. ×800.
Fig. 8. Germinating spores of fungus from Victoria (1). ×300.
Fig. 9. Petri dish culture of fungus from Victoria (1) on raisin agar.
Fig. 10. Petri dish culture of fungus from Pond's Seedling (7) on raisin agar.

STUDIES IN ENCHYTRAEID WORMS. *HENLEA FRAGILIS* FRIEND.

By the Rev. HILDERIC FRIEND, F.R.M.S.

(With Plates XXVII—XXXII.)

The object in view in preparing these Studies is to supply an accurate and detailed account of British White Worms or Enchytraeids. These researches have been carried out in the Department of Agricultural Zoology, Birmingham University, which is under the direction of Prof. Gamble, D.Sc., to whom the writer here acknowledges his indebtedness.

In the following pages the Genus *Henlea* is the subject of study, the type chosen being *Henlea fragilis.* The history of the genus is first set forth, and previous definitions examined. These are found to be unsatisfactory, and a new definition is proposed. The history of the type is then given, with a detailed account of the external characters and internal organs. This leads up to the definition of the species, and the discussion of its systematic position, which is shown to be related on the one hand to *Henlea hibernica* Southern, and on the other to the American species *Henlea moderata* Welch.

The group of worms known as Enchytraeids is a large and important one. While the species are, on the whole, known to be of great service in relation to Agriculture, there has for some time been a suspicion that in certain cases they are injurious to plants, and these studies arise out of efforts now being made to determine the question of their value. The family name *Enchytraeidae* is of interest in this connection. By its etymology (*ἐν, in* and *χύτρος, a flower pot*) we learn that the type was first observed in gardens, and might be regarded pre-eminently as the "pot worm." Later research, however, reveals the fact that the various species and genera included in the family enjoy the widest possible distribution in this country, being found not only in pots and flower borders, but in manure heaps, leaf mould and vegetable refuse of all kinds, from low tide mark on the seashore to the tops of our highest hills.

No fewer than 11 genera of Enchytraeids are now known to occur in Great Britain. Taken alphabetically they stand as follows: *Achaeta, Bryodrilus, Buchholzia, Chamaedrilus, Enchytraeus, Fridericia, Grania, Henlea, Lumbricillus, Marionina* and *Mesenchytraeus*. Of these, *Henlea* is the first in order of treatment by Michaelsen (6), and a typical British species of this genus has been chosen as the first representative of the family for treatment in this series of studies.

The genus *Henlea* was established by Michaelsen (5) in 1889, six species being then recognized. Though the number was so limited, the characters were somewhat heterogeneous. The main features were as follows: Setae not of uniform size or arrangement. Head pore between the prostomium and peristomium or first body ring (usually represented by the symbol 0/1). No dorsal pores. Blood colourless. Nephridia with the duct arising near the septum. Dorsal vessel arising in front of the clitellum. Oesophagus sharply marked off from the intestine.

All the characters here enumerated, however, may be found in one or other of the allied genera. In 1895 Beddard (1) discussed the definition, which was five years later further extended and modified by Michaelsen (6), who at this time admitted only five species as beyond dispute. He drew attention to the following points: Coelomic corpuscles (Lymphkörper, lymphocytes) of one form only, large, mostly discus-shaped, seldom elliptical, darkly granulated. The oesophagus merging suddenly in the intestine in the 7th, 8th or 9th segments. The origin of the dorsal blood vessel anteclitellian in segment 8 or 9, spermathecae lacking diverticula, but communicating with the oesophagus.

In the systematic arrangement the presence or absence of oesophageal glands (Darmtaschen, intestinal diverticula) finds place. The recent discovery of many new species both in Great Britain and abroad, however, shows that not one of the characters in the new definition is strictly generic. We meet with species whose coelomic corpuscles differ from those of the type, others in which the oesophagus does not merge suddenly but gradually into the intestine. In a few instances the spermathecae possess diverticula, and are merely attached to, but do not communicate with, the intestine. Finally, we have what seemed to be the most distinctive characters swept away; for certain species have been discovered whose dorsal vessel does not originate in front of the clitellum, and whose intestine is destitute of special glands (Darmtaschen).

Hence a new definition became necessary. There are many points which would serve the systematist in his endeavours to distinguish species, though it is very difficult, in the light of the most recent research, to define the genus. The brain, to which no allusion has been made in any of the foregoing diagnoses, is usually of quite a definite type, being as a rule hardly longer than broad, and more or less concave behind. The spermathecae vary; salivary glands (peptonephridia) may be present or absent, as may also the oesophageal glands. The setae may be of the *Fridericia* type, *i.e.* shortest in the middle of each bundle, or of equal length, and the oesophagus may or may not go sharply into the intestine. Sometimes there are glands at the ectal opening of the spermathecae in the intersegmental groove 4/5, at other times they are absent. The coelomic corpuscles may be discoid, as in the assumed type, or irregularly shaped; the salivary glands may or may not open into the intestine, while their position varies greatly. Sometimes they are dorso-lateral, at other times they are ventrally placed, while in a third group one gland is dorsal and the other ventral. Once again the dorsal vessel may arise in segments 7, 8 or 9, in which case we usually find the oesophageal glands near its point of origin, where also the oesophagus suddenly merges in the intestine; or the vessel may originate in or near the clitellum, the oesophageal glands being in this case wanting, while the oesophagus passes gradually into the intestine. When the oesophageal glands are present there may be only one (*Henlea moderata* Welch), or we may find a pair (*Henlea nasuta* Eisen). In at least one instance (*Henlea ventriculosa* D'Ud.) they number four (or two pairs).

Welch has well observed that "Taking the genus as a whole, there is a remarkable variation in the different organs. *Henlea puteana* Vejdovsky is unique in having two pairs of spermathecae. The species of the genus can be grouped in one of several ways according to the criteria, which may be the character of the setae, the presence or absence of intestinal diverticula (Darmtaschen or oesophageal glands), the presence or absence of peptonephridia (salivary glands), the place of origin of the dorsal vessel, or the presence or absence of diverticula[1] on the spermathecae." The nearest allies are *Buchholzia* and *Bryodrilus*. A newly described American species (*Henlea moderata* Welch) with its solitary oesophageal gland brings us very near to these genera, which are also closely approached by other species in other directions.

As a step towards a more satisfactory definition I suggested some time ago (4) that it would be well to form at least two groups, calling

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[1] The original reads "ampullae," but what we call "diverticula" are clearly intended.

the species which have no oesophageal glands *Henleanella*, and thus reserving the term *Henlea* exclusively for those species in which oesophageal glands exist. The forms which depart from these two divisions by showing the origin of the dorsal vessel in the region of the clitellum might possibly be arranged in a third group as *Henleana*. In the present paper, however, I do not propose to discuss these forms, but to confine myself purely to those species which, having oesophageal glands on or near the 8th segment, where the dorsal vessel takes its rise, may be regarded as true or typical species.

Henlea thus shorn of doubtful characters would reveal the following points:

1. *Generic*. Glands or diverticula (Darmtaschen or oesophageal glands) arising from the oesophagus in or near segments 7, 8 or 9, and varying in number from one (*H. moderata* Welch) to four (*H. ventriculosa* d'Ud.)[1]. Dorsal vessel anticlitellian, arising immediately behind the oesophageal glands. Oesophagus passing sharply into the intestine where the glands open into it.

Coelomic corpuscles (Lymphkörper) of one form, large, discus-formed or broadly elliptical, darkly granulated. Brain slightly longer than broad, concave or incised before and behind.

2. *General*. Setae straight or slightly curved within, varying both in number and length; often *Fridericia*-like, *i.e.* with the shortest in the middle of the bundle. Small head pore, situated between the prostomium and the first body segment or peristomium, and represented by the sign 0/1. Dorsal pores absent. Blood colourless; vascular system destitute of the so-called heart-body (Herzkörper), though the dorsal vessel is often enlarged in segments 6 to 9.

Nephridia with small anteseptal portion, the postseptal changing into the duct immediately behind the septum. Spermathecae consisting of a sac-like body, usually with a swollen portion or ampulla; glands at the opening in intersegment 4/5 sometimes present, diverticula rare (not yet found in any British species), the posterior portion of the spermatheca attached to the oesophagus or opening into it. Salivary glands present. Three pairs of septal glands in segments 4/5, 5/6 and 6/7. Sperm funnels in the 11th segment with duct ending in the penial bulb on the ventral side of segment 12. Oviduct aborted, the oviducal pore being usually invisible.

It will be seen that one or other of the foregoing general characters is shared by other genera, and that the line of demarcation is anything but sharp and clear. But it must at the same time be noted that if we limit the genus as suggested the oesophageal glands, coelomic corpuscles, and brain are distinct characters separating *Henlea* from every other genus. *Buchholzia* and *Bryodrilus* will then be the only genera at all likely to prove confusing and these can be distinguished by characters which are perfectly definite and satisfactory.

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[1] These glands are dorsal to the oesophagus, whereas in Kerria, for example, they are ventral.

If now we look at one of our indigenous species which approaches most nearly the typical form, and subject it to a very careful and systematic examination, we shall have a standard by which all the other species may be tested. A suitable type presents itself in the case of *Henlea fragilis* Friend, and we chose it partly on account of certain points of interest attaching to its life-history and distribution.

Henlea fragilis was first found by the author at Bopeep, St Leonards-on-Sea, on December 21st, 1911, against the walls of an arch through which a streamlet flows into the sea. Along with it were several other new species, which were described (3) in the *Journal Roy. Micros. Soc.* for 1912 (pp. 586–598). The claims of this species to special recognition lie, not alone in its typical character, or in the fact that I have been able to obtain abundance of living material for microscopic investigation, but also in the following curious coincidence.

In the summer of 1914 a box containing living specimens of Peripatus for research was received at the Birmingham University. As it was found on examination to contain a large number of Enchytraeids, Professor Gamble, F.R.S., kindly handed the material over to me for investigation. The white worms proved to belong to various species and genera, and among them were several finely developed and fully mature specimens of *Henlea fragilis*. The question, how the worms, which had been found by me previously in Sussex, came to be included in earth from Cape Town is one which may be discussed elsewhere. It suffices to say that I was in possession of excellent material both for the study of the living worm and for the preparation of sections, and could therefore produce a fuller and more detailed account of *Henlea* than had hitherto appeared.

In February, 1915, Mr Cox, Steward in the Department of Zoology at the Birmingham University, prepared for me an excellent series of sections both transverse and longitudinal-vertical, and it is upon the study of these sections, carefully checked by a constant reference to the living worm, that the following details are based. The illustrations which accompany the paper have all been made from the microscopical sections by the aid of Zeiss and Leitz objectives and camera lucida. The worms were kept for a time in damp blotting paper before being killed, in order to clear the intestine and render the making of sections both safe and easy.

1. *External characters of Henlea fragilis.*

An enchytraeid worm with colourless blood, closely related to *Buchholzia* and *Bryodrilus*, of normal size and appearance with an average length of 12–15 mm. and possessing about 50 segments. Each segment save the first and last bears setae (Borsten), but those found on segment 12 disappear as the adult stage is reached and the girdle begins to develop. Apparently the smallest and innermost setae in the ventral bundles of segment 12 are those which hold on most tenaceously, and are the last to disappear: a point which may not be without significance. The setae are arranged in four bundles on each segment (Plate XXVII, fig. 1) and are disposed, not at equal distances around the body, but on the lateral and ventral surfaces. They vary in number in different specimens and at different times. The largest number yet recorded for any bundle is eight, and so high a number has only been found rarely and in perfectly adult forms. There may be as few as two in a set, particularly in immature specimens, and the size varies as well as the number.

The head is nearly oval in longitudinal section, showing a slight but distinct depression in the middle region (Plate XXXII, *dep.*). Between the prostomium and the first body segment the head pore (*h.p.*) is seen. It lies in the intersegment 0/1, but is not easily found in the living worm, though its presence may be readily detected by the stream of coelomic corpuscles which is forced out under the pressure of the cover glass. It communicates directly with the coelom, and gives relief to the body contents. Its close proximity to the brain might suggest that it serves specially to prevent congestion in that region. There are no other pores on the dorsal side.

Vacuolar or glandular cells occur at intervals in the body wall. They are neither so large nor so numerous as those which are found on the girdle, and in neither instance are they arranged in definite rows, as is the case with some of the Enchytraeids. These cells are usually more numerous on the dorsal than on the ventral surface.

The clitellum is exceedingly glandular (Plate XXVII, fig. 2 *A*), the vacuolar cells extending right round the girdle when the adult stage has been perfectly attained. The whole of the 12th segment, with portions of segments 11 and 13, is taken up by the clitellum or girdle. Underneath the vacuolar or glandular layer is another composed of muscles arranged longitudinally, which extend throughout the entire length of the body. A very thin cuticle covers the outer muscular layer. The anus is terminal, and is usually very glandular.

2. *Internal characters of Henlea fragilis.*

Nervous system. The brain lies in segments 1 and 2, and in longitudinal vertical sections is oval in outline (Plate XXXII, *br.*). Viewed dorsally, however, the brain appears in the living worm somewhat longer than broad (Plate XXIX, fig. 3), nearly or quite straight in front and concave behind. At the same time it must be noted that the posterior margin is liable to considerable modification, and may appear to be straight or even convex in fully adult specimens. It is composed of two kinds of cells, as is also the nerve cord. Two stout strands are given off anteriorly, and these bend down in the front part of the first segment, in the ventral portion of which they combine to form the nerve in segment 2. The structure of the nerve is best seen in transverse sections (Plate XXVII, fig. 1, *n*). The shape of the brain is instructive when compared with that of *Fridericia*, which almost invariably has decidedly convex extremities and is oval in shape as well as in longitudinal section. Beddard has truly remarked that "the form of the brain in these worms is often highly characteristic of the genus or species."

Coelomic corpuscles. These bodies (Lymphkörper or lymphocytes) are very large and conspicuous. Their size in relation to the vacuolar glands of the girdle may be seen by reference to the illustration (Plate XXVII, fig. 2 *B*), while the other figures supply opportunities for comparison with other portions of the system. They agree with those of *Henlea moderata* Welch (8), and are almost round or of a broad elliptical shape. Too much stress must not be laid, as Welch seems to do, on their unequal distribution in the coelom, as they move freely from segment to segment, and though not usually so abundant in the anterior portion of the body they nevertheless have free access to all the front segments, as is shown by the way in which they stream from the head pore when the animal is affected by the pressure of a cover glass on the microscopic slide. The coelom itself calls for no special notice.

Alimentary system. It will be convenient under this head to discuss a variety of organs, including the pharynx, tongue, oesophagus and oesophageal glands, septal and salivary glands and intestine, in the order in which they occur in longitudinal section. The mouth lies, as usual, on the ventral side of the prostomium (Plate XXXII, *mo.*) with the peristomium or first body segment as a lower lip. Behind the intersegment 1/2 lies a taste organ (Plate XXXII, *t.o.*) or tongue. It arises from the floor of the buccal cavity, and under certain conditions has exactly the appearance of a valve. It projects into the pharynx, and is capable of being moved forwards and backwards. The base of the organ is broad, and the free end pointed. This gives it the outline in longitudinal section of a short curved wedge. A pair of minute processes (not shown in the illustration) may be seen laterally in the posterior region. In view of the fact that the number and arrangement of these organs vary with the species, and may be solitary, paired or even quadrupled, their study is of considerable interest and importance.

The pharynx is situated in the 3rd segment (Plate XXXII, *ph.*) and has a strong dorsal infolding. It agrees in form and structure with that of *Enchytraeus pellucidus* Friend, as described by Stirrup (7). There is no trace of a stylet, neither can I find any evidence of a direct connection between it and the septal glands. The muscles (Plate XXX, *p.m.*) are very strongly developed. Of the septal glands there are three pairs, which, by reason of their staining readily, are conspicuous objects in longitudinal sections. They each consist of two or three unequal lobes (Plate XXX, *s.g.*), the largest of which is dorsally placed, and posterior to the smaller. Their form and appearance may be best judged by the illustration. It will be well to observe that though the septa appear to be wanting in the first four segments, those in 5/6, 6/7 and 7/8 are strongly developed ventrally (Plate XXX, *t.s.*) in order to form a basis or support for the glands, which project forward, and, like the nephridia, each occupy portions of the two segments 4/5, 5/6 and 6/7.

The next organs attached to the alimentary tract which arrest our attention are the peptonephridia or salivary glands. These are developed, one on the dorsal, the other on the ventral surface of the oesophagus (Plate XXXII, *d.s.g.* and *v.s.g.*). Some authors treat them as part of the excretory system, but their use is still questionable, and we therefore prefer to notice them here. They do not appear to open into either the oesophagus or the pharynx, but are apparently blind appendages to the former, extending from the 4th to the 7th segments. The ventral salivary gland is closely attached to the under surface of the oesophagus, and is possessed of a strong outgrowth in segment 4 which lies in the coelom in near proximity to the nerve cord. It pushes back the septum between the fourth and fifth segments, but does not normally pass into the latter segment with the rest of the organ. Tubules are given off at intervals, and the posterior extremity is branched.

The dorsal salivary gland commences in front of the first septal gland (Plate XXXII, *d.s.g.*) and lies between that body and the oesophagus. It then passes posteriorly along the coelom attached dorsally to the oesophagus, and ends in two or more small branches (Plate XXXII, *b.s.g.*) in the 6th and 7th segments. Though both salivary glands lie in such close proximity to the oesophagus neither has been observed to enter into it. The condition described above is very similar to that which Welch has so clearly set forth in his account of *Henlea moderata*. So far as I am aware these two species, together with *H. urbanensis*, are the only ones in which salivary glands of this type have yet been described. The peptonephridia are certainly very valuable for purposes of diagnosis on account of their great variation in shape, size and position, and will in future play a more important part in both generic and specific description. It should not be overlooked that in this species, nephridia are found in the segments (6 and 7) which contain peptonephridia. (See Beddard I. 47.)

The oesophagus, whose lumen is ciliated, gives rise in segment 8 to a pair of organs known as oesophageal glands or intestinal diverticula (Darmtaschen). These are the chief distinguishing features of the genus, and merit more than a passing study (Plate XXX, *oes.g.*). They are attached to the oesophagus and open directly into it at the point where the latter enters the intestine. They are somewhat heart-shaped, and consist of a number of tubules, arranged irregularly round a central cavity or duct (Plate XXVIII, *cav.*). In *Henlea* as above defined they are always present, but vary in number and position. In *H. moderata* Welch there is only one gland, which tends to relate it to *Buchholzia*, while in *H. ventriculosa* there are four. Usually, however, there are two, and these lie between the 7th and 9th segments. The latest account of these structures is by Welch (8) who complains that most descriptions are very meagre. It may be useful, therefore, if we give some further details of their structure in *H. fragilis*. The glands, though originating in segment 8, are not strictly limited thereto, nearly one-fourth of the organ projecting through the septum into the posterior portion of segment 7. The anterior extremity is more pointed at the front than the section first chosen for illustration (Plate XXX) suggests. The interior tubules are made up entirely of one form of cell, and the lumen is ciliated. Surrounding the whole is an outer layer which shows no definite structure, but is filled with minute dark-staining points (Plate XXVIII). In this species there are no chloragogen cells as shown by Welch in *H. urbanensis*. The dorsal blood vessel may be seen (Plate XXVIII, *b.v.*) immediately behind the diverticulum, but in front of the septum which it pushes back into the coelomic cavity of the 9th segment.

The intestine commences in segment 9, and is only distinguished from the oesophagus by its greater diameter (Plate XXX, *int.*).

Vascular system. The blood vessels of the Enchytraeids are normally few, and the arrangement is simple. There is a dorsal vessel which arises either in front of the clitellum (preclitellian), within that organ (intraclitellian) or behind it (postclitellian). In all the Henleas as at present defined the dorsal vessel arises immediately behind the oesophageal glands in or near the 8th segment. In *Henlea fragilis* it sometimes appears as if it originated in the anterior portion of segment 9: but sections show that it is wont to push back the septum 8/9 (Plate XXXII, *sep.*) in front of which it commences (Plate XXX, *d.b.v.*). In segments 8, 7, 6 there are enlargements of the vessel, that in 8 (Plate XXXII, *d.b.v.*) being about twice as large as the one in segment 7, which in its turn is of greater dimensions than the one in segment 6. Each vessel contains a substance which coagulates and stains readily (Plates XXVIII and XXXII, *b.v.*). It cannot be seen in the living worm on account of its transparent nature and the absence of a colouring medium. Observations made on sections of a similar character taken from the red-blooded Enchytraeids, however, show that this is the blood-plasm. The dorsal vessel passes forward to the head, giving off three commissures on the way, dips under the brain and bifurcates, so as to form the two anterior branches of the ventral vessel. The disposition is normal and calls for no further description.

Sexual characters. The sexual organs consist of ovaries and testes, together with numerous accessories, such as sperm funnels and ducts, penial bulbs, male and female apertures, and the storing chambers or spermathecae. We begin with the latter as being the first organs which meet the eye when passing from the head backwards.

The spermathecae (Plate XXIX, fig. 1) of *Henlea fragilis*, of which one pair exists, are situated as usual between the first and second septals in the fifth segment, the opening being in the intersegment 4/5, which is destitute of glands. There are no diverticula, but an ampulla is found about mid-way between the two extremities. The posterior and internal portion of each spermatheca is embedded in the epithelium of the oesophagus (Plate XXIX, fig. 2, *sp.*) but does not open into the digestive tract, as it is said to do in other species. It simply ends blindly in the tissues of the intestinal canal. Neither do the posterior extremities of the two spermathecae join each other as is the case, for example, with those of *H. urbanensis* Welch and *H. moderata* Welch.

The sperm funnels (Plate XXXI, *f.*) whose mouths are usually filled with masses of spermatozoa lie in the 11th segment. They are only slightly longer than broad and are not perfectly symmetrical in shape, the portion lying towards the dorsal side of the body being enlarged. The collar is moderately large and slightly curved, and surrounds the opening to the duct which passes through the septum into segment 12. Here the duct is long and coiled (Plate XXXI, *d.f.*), but, unlike that of *Enchytraeus pellucidus* Friend and some others, is confined to one segment. It ends in the penial bulb (Plate XXXI, *p.b.*); an organ which has been the subject of careful investigation by Eisen and others. The relative size of the penial bulb can be easily judged by its relation to the sperm funnels in the figure. It is small in comparison with that of some other Enchytraeids and belongs to the group which Eisen regards as lumbricillid in type. It is composed of two kinds of cells and the sperm duct passes through it centrally.

The testes originate in the posterior side of septum 10/11, by which means they are placed in the anterior portion of the 11th segment, in close proximity to the sperm funnels. Here may be found in the fully adult worm, enormous masses of spermatozoa (Plate XXXI, *sper.*) and in good sections the entire process of spermatogenesis can be readily traced. Passing from the male organ in the form of intensely minute spheres they rapidly develop and congregate around the mouth of the sperm funnel, through which they pass to the aperture in segment 12.

The ovaries lie in the adjoining segment, being attached to the ventral portion of the septum 11/12. The developing ova are pushed off and lie in the coelom of segment 12, but the oviduct and its pore are aborted, and every attempt to find out how the eggs are deposited in the cocoon has hitherto resulted in failure.

Nephridia are present in segments 6/7 to 10/11, and again behind the girdle. They are of the usual Enchytraeid type with a small portion in front of the septum and a larger portion (postseptal) behind, which contracts to form the duct immediately behind the septum. The duct and postseptal are about of equal length (Plate XXXI, *neph.*).

Chloragogen cells. It is a usual thing for Enchytraeids to possess certain special cells on the outer surface of the intestine. As a rule these cells commence about the fifth segment, and extend to the posterior extremity of the body, save that it is no unusual thing for their number to be greatly reduced in the region of the girdle. In this respect *Henlea fragilis* shows a decided departure from the rule, and the absence of chloragogen cells from the first twenty to five-and-twenty segments is a marked characteristic. I have not found them in any instance in front of segment 20. We may now sum up the main points.

Definition of Henlea fragilis.

Length about 15 mm. Segments 55 to 60. Setae 4 to 8, the innermost in each bundle usually the shortest. Girdle extending over one-third of segment 11, the whole of the 12th segment and two-thirds of segment 13. Brain slightly longer than broad; spermathecae without glands or diverticula. Three pairs of septal glands; one pair of oesophageals in segment 8, behind which, in the same segment, the dorsal vessel arises. Nephridia with small anteseptal portion; duct immediately behind the septum as long as the postseptal. Sperm funnels only slightly longer than broad, the duct not centrally placed, penial bulb of moderate dimensions of the lumbricillid type. Coelomic corpuscles large, broadly elliptical; chloragogen cells commencing behind the 20th segment. Salivary glands present, one dorsal, the other ventral, attached to the epithelium of the intestine in segments 4 to 7, branched at the ends.

Observations made on a large number of specimens in various stages of development show the following among other points of interest:

(1) The number of setae seems to increase with age.

(2) The ampulla of the spermatheca appears to enlarge as the adult stage is reached.

(3) The brain tends to become convex posteriorly as the worm grows older.

(4) The salivary glands undergo modification as the animal develops.

Systematic position of Henlea fragilis.

It is not an easy matter to assign to this species of *Henlea* its rightful place. We have agreed, however, for the present, to eliminate all species which do not possess oesophageal glands (*Henleanella*), as well as those species (*Henleana*) whose dorsal vessel is of intraclitellar origin. This reduces the number of genuine Henleas known to be British (3) to 10 species, viz. *attenuata, fragilis, fridericioides, heterotropa, hibernica, nasuta* (= *leptodera*), *pusilla, quadrupla, triloba* and *ventriculosa.*

These species vary in relation to the number, shape and arrangement of their setae, the character of their coelomic corpuscles, the presence or absence of glands to the spermathecae at the opening between segments 4 and 5, the number and position of the taste organs or tongues, the salivary glands, the shape and dimensions of the sperm funnels, the nature of the penial bulb, and the point of origin of the dorsal vessel. In *H. ventriculosa* two pairs of oesophageal glands are present, the other nine species having one pair only. *H. fridericioides* possesses glands to the spermathecae, which also sometimes occur in *H. hibernica*; but as a rule they are absent. Diverticula have never yet been found on the spermathecae of British species. In *H. quadrupla* there are four pairs of septal glands while as a rule three pairs only are present. On the whole *H. fragilis* approaches Southern's *H. hibernica* more nearly than any other British species. This may be shown as follows:

	H. fragilis Friend.	*H. hibernica* Southern.
Length	15 mm.	15–20 mm.
Segments	55–60	55–60
Setae	4–8	5–9
Oesophageal glands	One pair in seg. 8	One pair in seg. 8
Dorsal vessel	8/9	8/9
Contractile swellings	6, 7, 8	6, 7, 8

The brains are also of the Henlean type. But while *hibernica* is opaque, *fragilis* is transparent; *hibernica* often has small spermathecal glands which are not found in *fragilis*; the sperm funnels of *hibernica* are three or four times as long as broad, and the pair of salivaries are ventral, whereas in *fragilis* one is ventral and the other dorsal, while the sperm funnels are only slightly longer than broad. If we look particularly at the salivary glands we have to go to America for a case similar to that of *H. fragilis*. While no other true British *Henlea* has yet been found whose salivary glands are so arranged, Welch (8) has recently described species (*H. moderata* and *H. urbanensis*) which show the same feature. But in the former four taste organs are present, there is only one oesophageal gland, and the dorsal vessel arises in the 9th segment, while in the latter there are two taste organs and the dorsal vessel again takes its rise in segment 9. For the present therefore we are content to allow *H. fragilis* to stand related to *H. hibernica* on the one hand and *H. moderata* and *H. urbanensis* on the other.

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EXPLANATION OF PLATES XXVII—XXXII

Plate XXVII, fig. 1. Transverse section through segment 5.
,, ,, fig. 2. Girdle glands (*A*) and coelomic corpuscles (*B*).
Plate XXVIII. Oesophageal gland.
Plate XXIX, fig. 1. Diagram of spermatheca.
,, ,, fig. 2. Transverse section showing spermathecae ending blindly.
,, ,, fig. 3. Dorsal view of brain, in outline.
Plate XXX. Long. vert. section through segments 4–11.
Plate XXXI. Long. vert. section through segments 9–15.
Plate XXXII. Long. vert. section through segments 1–9.

A. Glands in girdle segment. See *v.g.*
B. Coelomic corpuscles to same scale. See *c.c.*
I–XV. Segment numbers.

a.s. ampulla of spermathecae; *br.* brain; *b.s.g.* branches of salivary glands; *b.v.* blood vessel; *cav.* cavity in oesophageal gland; *coel.* coelom; *c.c.* coelomic corpuscles; *cil.* cilia of intestine; *d.* dorsum; *dep.* depression in dorsal portion of head; *d.b.v.* dorsal blood vessel; *d.f.* duct of sperm funnel; *d.s.g.* dorsal salivary gland; *f.* funnel; *g.* girdle; *h.p.* head pore; *int.* intestine; *l.s.s.* lateral setae sac; *m.* muscles; *mo.* mouth; *n.* nerve cord; *neph.* nephridium, *oes.* oesophagus; *oes.g.* oesophageal gland; *p.b.* penial bulb; *ph.* pharynx; *p.m.* pharyngeal musculature; *pr.* prostomium; *sep.* septum; *s.g.* septal glands; *sp.* spermathecae; *sper.* spermatozoa; *t.o.* taste organ; *t.s.* thickened septa to carry glands; *v.* ventral surface; *v.g.* vacuolar glands; *v.s.g.* ventral salivary gland; *v.s.s.* ventral setae sac.

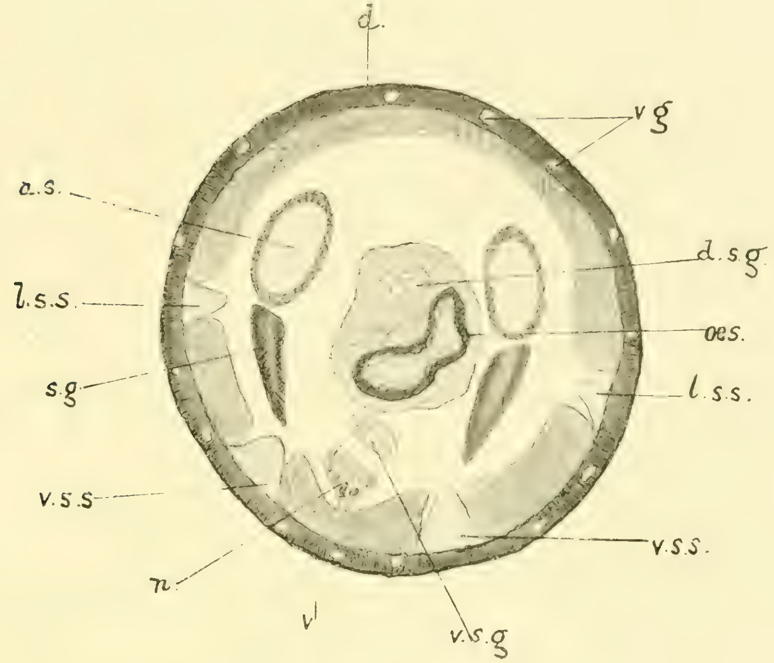
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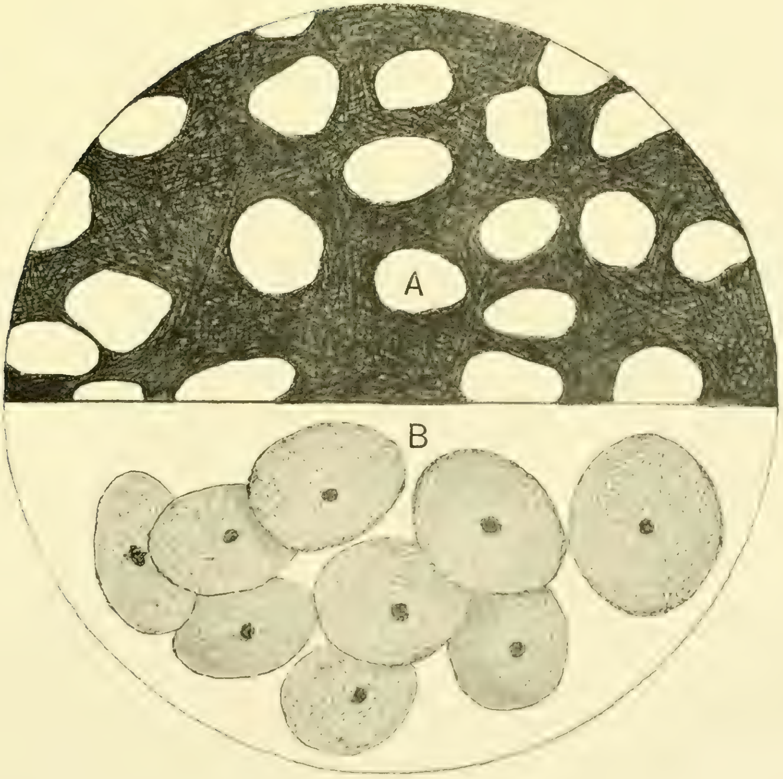
Fig. 1



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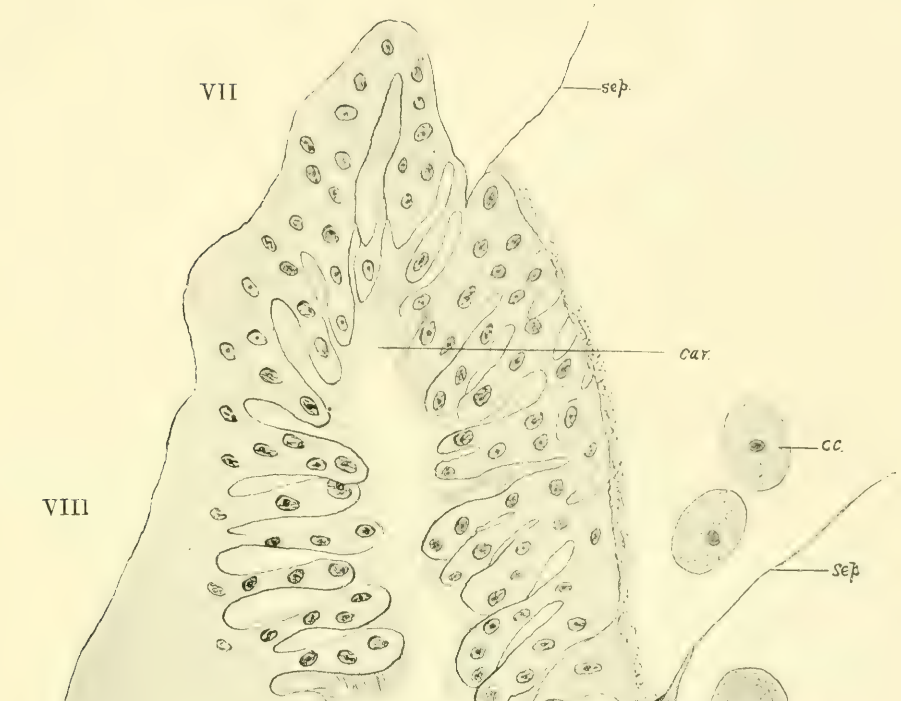
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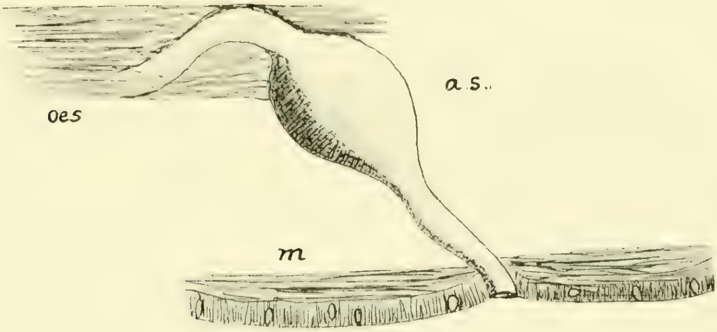

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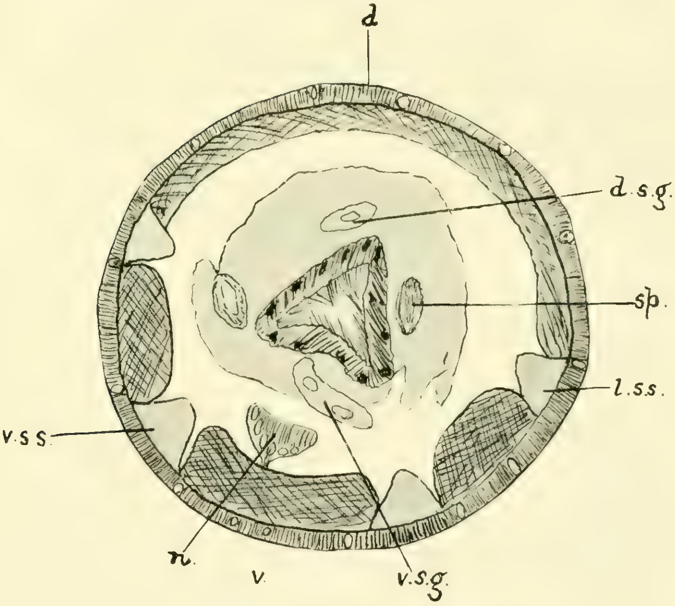
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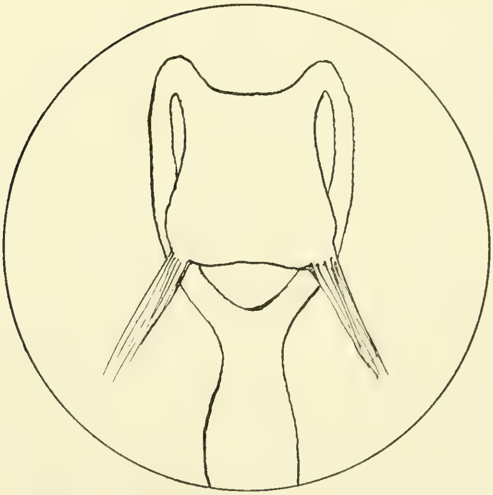

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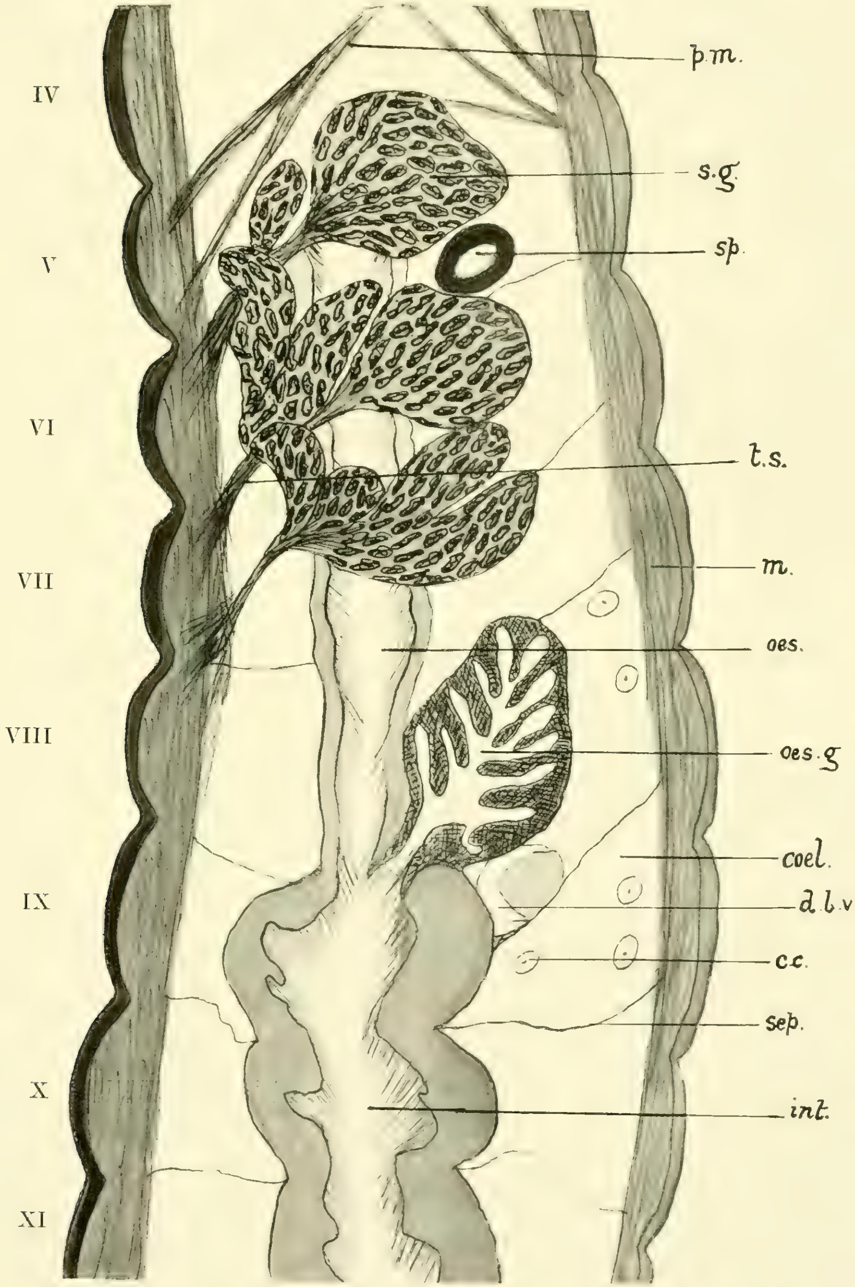
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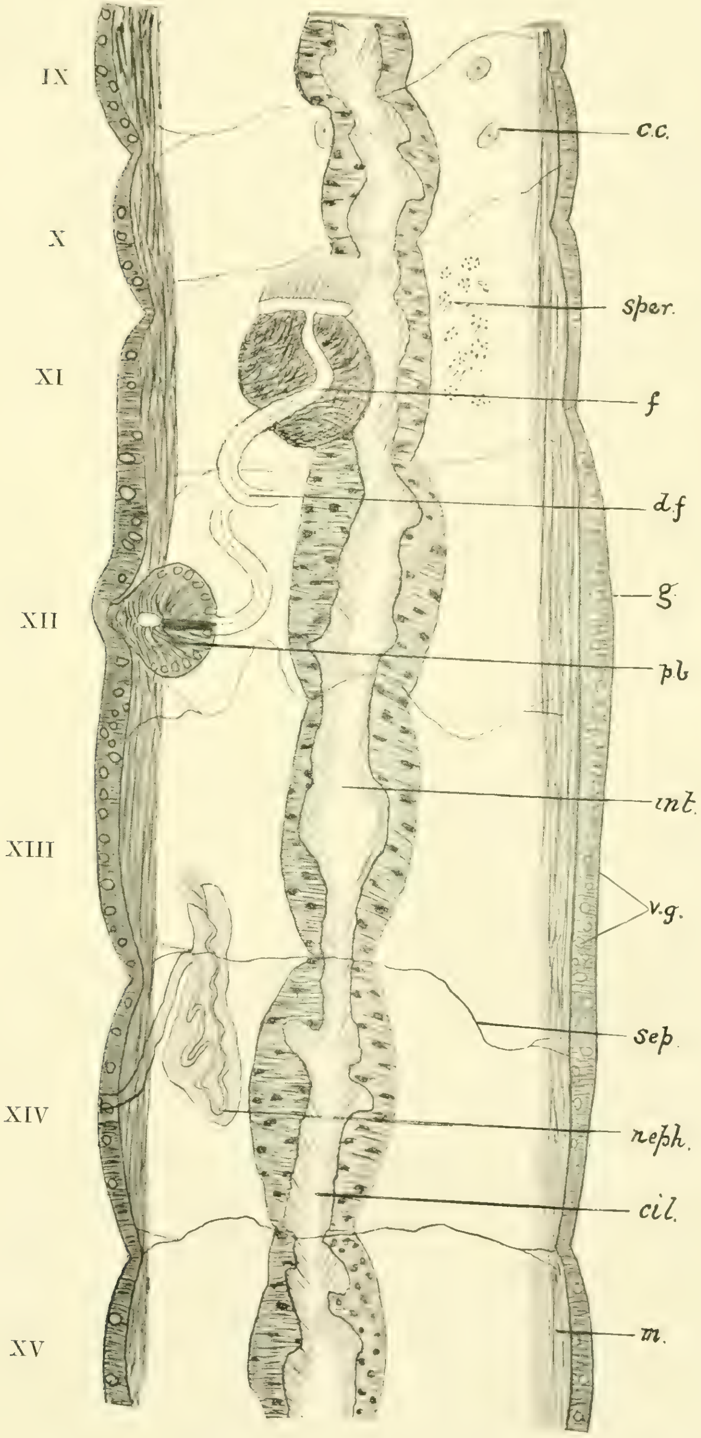
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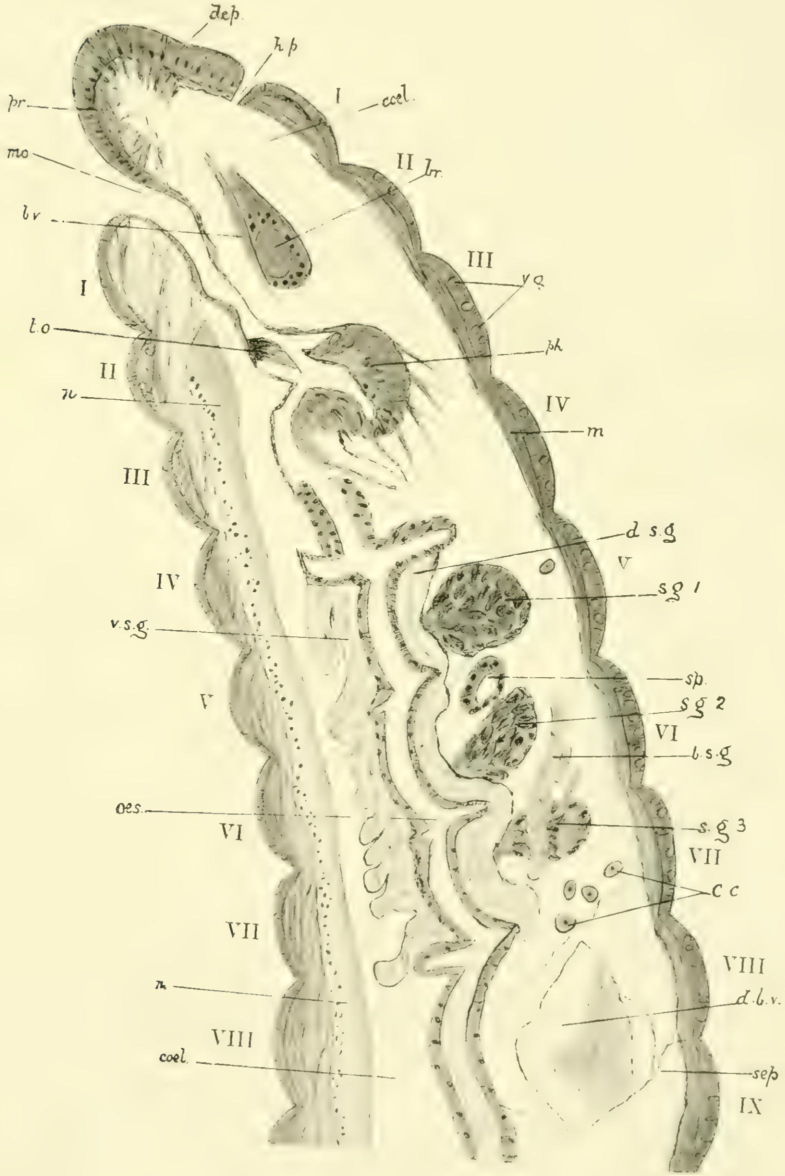
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IX

OBSERVATIONS ON SOME DISEASES OF PLANTATION RUBBER IN MALAYA.

BY F. T. BROOKS, M.A.,

Senior Demonstrator of Botany, Cambridge University; sometime Government Mycologist, Federated Malay States.

(With Plates XXXIII—XXXV.)

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1. *Introduction.*

THE present paper is an account of observations made upon some diseases of plantation rubber while the writer was acting as Government Mycologist in the Federated Malay States during 1914. Accounts of Pink Disease caused by *Corticium salmonicolor* and of the disease caused by *Ustulina zonata* have been published in separate papers (5, 7) and are only briefly mentioned here.

Generally speaking, Para rubber is a particularly healthy tree not only in its home in the valley of the Amazon but also in Ceylon, Malaya, Borneo, Java, and Sumatra, where such enormous areas have been planted with it that the plantation industry is superseding the collection of rubber from wild sources. In Malaya large numbers of estates are contiguous to one another, and in travelling by rail from Penang to the borders of Johore one passes through an almost continuous rubber forest, broken here and there by other cultivations or by tin-mining areas and only once interrupted by a considerable jungle belt. These estates are usually devoid of inter-crops, hence this enormous tract of country, planted with a single product, would offer favourable opportunities for the establishment of disease on a serious scale were it not for the robust nature of *Hevea brasiliensis*, its relative immunity from disease, and the measures already taken to check fungoid parasites. Fortunately no disease of an epidemic nature has yet attacked plantation rubber with the possible exception of Pink Disease (7), which sometimes affects numbers of trees simultaneously in certain parts of the country during wet weather. It is probable, however, that Pink Disease can be kept under control if proper precautions are taken. No serious leaf disease of *Hevea* has yet been recorded in the East and the type of leaf, thin but tough, is one not specially liable to fungoid attack. One can safely say that plantation rubber is at present at least as healthy as most of the agricultural crops of temperate countries.

Although plantation rubber has been so healthy hitherto it must be emphasised that this happy state can only be maintained by continued vigilance in the treatment of disease as soon as it appears. The planting of enormous areas with one kind of plant offers special facilities for the propagation of disease unless any danger which threatens is dealt with drastically at once. It is fortunate for the plantation rubber industry that planters as a body are keen to combat disease and it is now the rule on estates to seek expert assistance as soon as troubles of this nature arise. Planters have by experience developed sound views on the subject of plant sanitation. The ravages of *Hemileia vastatrix* and other injurious organisms upon coffee in Ceylon has made a deep impression upon them. Many Malayan planters were formerly planters in Ceylon where they had actual experience of the effect of disease in the coffee industry or heard about it from the older men, and not a few saw similar ravages upon coffee in the Federated Malay States before the cultivation of this crop on a large scale was abandoned.

Within the last few years estate practices have changed in a direction that conduces to maximum vigour in the trees. Whereas it was formerly customary to plant 350 and more trees to the acre it is now usual to plant only about 100 per acre. On many older estates thinning out has reduced the number of trees per acre to 80 or 90. Now that overcrowding is prevented the trees have a better chance of attaining their natural habit of growth, and air and light being freely admitted to all parts of the tree there is less danger of the establishment of disease. Where estates are thinned out it is necessary for the rubber stumps to be removed from well below the surface of the ground, otherwise there will be danger of root troubles later.

The methods of tapping in vogue at present are much more conservative than those formerly practised and longer periods are allowed for bark renewal, all of which tends to preserve the health and vigour of the trees. Many of the older trees were overtapped during the boom of 1910 and in consequence are poor specimens at the present day.

On account of the reduction in the number of trees per acre on most estates in comparison with the number formerly customary, each tree is now of more value as a potential or actual latex producer, for in the long run an acre bearing 100 trees will yield more rubber than an acre in which 350 trees remain. An estate containing 80 to 90 trees per acre cannot afford to lose many trees through disease, hence the smaller the number of trees per acre the more important does it become to do everything possible to ensure vigour in the trees and, if disease does appear, to treat it as soon as possible. The retention of only 40 trees per acre has been advocated by some authorities but the loss of a few of these trees from disease would cause such a serious reduction in the output of latex that upon this consideration alone it would appear preferable to retain about 80 trees to the acre.

When trees are killed by disease in plantations more than two or three years old it is usually not worth while to replant vacancies as supplies become crowded out by the older trees unless the vacant areas are extensive.

It is found advisable on most estates to keep a special coolie gang to deal regularly with pests and diseases, the size of the gang being regulated according to the amount of work to be done. Upon estates affected by Pink Disease the maintenance of a pest gang sufficiently large to go over the whole estate once in three or four weeks is an imperative necessity. European supervision should always be exercised over the treatment of disease although coolies often become remarkably expert in finding unhealthy trees.

As is only natural, managers of estates sometimes express regret at the cost of the pest gang though many of them realise that money so spent is in the nature of insurance for the future. The welfare of the plantation rubber industry is dependent upon many factors, but it is obvious that without health in the trees this flourishing industry would cease to exist.

2. *Fomes lignosus*, Klotzsch.

This fungus which is the commonest cause of root disease of *Hevea* in Malaya is better known under the name, *Fomes semitostus*. Petch (11), however, has pointed out that *Fomes semitostus* proper is an entirely different fungus and that the correct designation of the fungus causing root disease of *Hevea* is *Fomes lignosus*, Klotzsch.

Soon after the establishment of rubber estates upon an extensive scale in Malaya this fungus became particularly troublesome in young plantations. As is well known it begins to grow upon the stumps which remain after the operations involved in planting up jungle land, and spreads thence by means of thick, yellowish white, mycelial strands which travel underground to the roots of young rubber trees. When the mycelium reaches the collar of the tree and spreads around it the tree dies.

Until recently the only mode of treatment for this disease used on a large scale lay in the destruction of affected trees and the isolation of diseased areas by means of trenches, but according to Richards (12) and Colenbrander (9) considerable success has been obtained by treating the roots of trees in the early stages of attack in the following manner. All trees are examined by opening the soil around the collar and if traces of mycelium are found the roots are fully exposed. Dead portions of roots are cut off, the external mycelium around living parts is scraped away, and the surfaces are then covered with a thin Bordeaux paste. The surrounding area is dug, all mycelial strands and dead wood being burnt. Richards (12) states that 75 per cent. of the trees affected on some estates have been successfully treated in this manner. A method similar to the above, but in which carbolineum is used instead of Bordeaux mixture, is described by Rutgers and Arens (16) as being used in Sumatra by Ris.

Since *Fomes lignosus* has been recognised to be a serious root parasite it has become customary on some estates to clear the land of jungle stumps and other timber a few years after planting. The cost of clearing varies greatly in different districts and depends largely upon the relative heaviness of the jungle that formerly covered the ground. Some of the stumps of old forest trees are so large and so resistant to decay that it is very expensive to remove them. Where, however, stumping can be carried out at a low figure before the estate comes into bearing the operation insures against serious attacks of root disease and facilitates the movements of coolies engaged in weeding and tapping. If the cost of clearing the timber is high, and *Fomes* does not threaten seriously, the cheapest course will probably be to treat root disease as it appears.

Even on estates which are apparently equally encumbered with timber the prevalence of *Fomes* varies greatly. Some plantations are seriously troubled by it in the early years, others are never much affected. Soils which are either very loose or clayey seem to favour the disease, soils of an intermediate character being less liable to it. The underground strands of the fungus are sometimes found at a depth of two and a half feet in loose soils but usually they are not more than 18 inches below the surface.

As Petch (10) points out, trees that are invaded by white ants have generally been previously attacked by *Fomes lignosus*. When such trees have been blown over, examination often fails to reveal signs of the fungus which together with much of the diseased wood has been consumed by the termites.

Even though the stumps and underground timber are not removed after planting, most estates which are properly managed become practically free from *Fomes* as they become older. This is due to the preference shown by *Fomes lignosus* for living upon timber in the early stages of decay; as the wood becomes more and more rotten there s less likelihood of *Fomes* growing upon it. *Fomes lignosus* comparatively rarely attacks old rubber trees and the death of many old trees attributed to it is likely to have been really caused by *Ustulina zonata* as described by the writer (5) elsewhere, or by *Sphaerostilbe repens* as will be pointed out later in this paper.

With the general decrease of this disease the fructifications of the fungus are now more rarely seen. The growth of lalang grass in badly kept estates appears to favour the development of fructifications at the base of diseased rubber trees, and upon one low-lying estate seen by me the fruit bodies were produced in abundance along the sides of the drains.

A large number of fructifications were examined by the writer at different times of the year and the large majority of them were found to be sterile. Sections of fructifications which were apparently mature repeatedly failed to show functional basidia. I never succeeded in obtaining a spore deposit, for even when sections showed that spore-producing basidia were present their number was very small. In view of the apparent paucity of spore production it would be interesting to have fuller information concerning the manner in which the fungus first begins to spread in a young rubber plantation.

3. *Polyporus rugulosus,* Lév.

On several occasions another polyporoid fungus was seen growing at the collar or upon exposed lateral roots of diseased rubber trees and it seemed likely that this fungus was the cause of the disease from which the trees suffered, although inoculation experiments are needed to settle this point definitely. The tissues of the host near the fructifications were invariably decayed, the foliage of the affected trees became thin, and the branches died back after the manner of trees attacked by a slowly growing root parasite. I saw this disease only in trees which were in tapping and it appeared to be more frequent in badly-drained low-lying estates than upon undulating land. One tree severely attacked by this fungus had been previously invaded by white ants.

The fructifications of this fungus are often densely imbricate and, in the aggregate, form large masses several inches across although a single pileus is only an inch or two in diameter. The upper surface is smooth, brownish, and zoned; the under pore-bearing surface is white when young, becoming yellowish brown with age; the pores are minute; the substance of the fructification is thin and although fleshy when young is leathery at maturity. Both in the colour of the pores and in the much thinner substance the fructifications of this fungus differ markedly from *Fomes lignosus.*

I am indebted to Miss Wakefield of the Royal Botanic Gardens, Kew, for kindly identifying this fungus as the *Polyporus rugulosus* of Léveillé. The type specimen of this fungus was obtained from tree trunks in Java and was described by Léveillé in 1844. Saccardo (17) has since placed the fungus in the genus *Fomes,* but on account of the texture of the fungus when young it is preferable to retain the original name. I have been unable to find any previous record of this fungus upon rubber trees.

Pending a further investigation of this fungus, rubber trees affected by it should be treated as for *Fomes lignosus.*

4. *Sphaerostilbe repens*, B. and Br.

During 1914 a considerable number of rubber trees were found to be affected by the fungus *Sphaerostilbe repens* which attacks the root system and advances upwards into the lower part of the trunk. The only previous record of this disease of rubber in Malaya was made by Richards (12) who mentions its occurrence in his report for 1912–13. According to the experience of the present writer this disease is by no means rare in older rubber planted on low-lying land in Malaya; it has been found in Northern Perak, in the district around Teluk Anson, and in the coast lands of Selangor. Only a few trees on undulating land were seen to be affected by it. The disease was first found on rubber estates in Ceylon in 1907 by Petch (10), who states that it is not confined there to the low country.

The foliage of rubber trees affected by *Sphaerostilbe repens* becomes thin and the branches gradually die back. The progress of the fungus being slow, a considerable time may elapse before the whole of the collar or all the lateral roots are affected and the tree succumbs.

If the roots of a tree affected by *Sphaerostilbe repens* are examined, the disease can be readily distinguished from the troubles caused by *Fomes lignosus* and by *Hymenochaete noxia* on account of the absence of external mycelium and by the presence of characteristic mycelial strands or rhizomorphs between the bark and the wood. These strands are usually flattened, are about $\frac{1}{8}$ inch in diameter, and vary in colour according to age from grey to dark brown or black; they are spread irregularly between the bark and the wood and sometimes occur also in the bark (cf. Plate XXXIII, fig. 1).

Even when the rhizomorphs have decayed, their former position is indicated by the presence of corresponding dark lines on the surface of the wood. I did not observe any tendency for these strands to spread independently through the soil. The affected trees were not usually contiguous to one another. The finer mycelium of this fungus permeates all parts of the bark and wood of the affected roots, the wood becoming discoloured and the bark often assuming a bluish purple colour when cut open. The roots of rubber trees affected by *Sphaerostilbe repens* often have a particularly foul smell, but this may be a secondary phenomenon induced by other agents of decay. The fungus advances up the tap root or along the laterals to the collar of the tree. On several occasions I observed it making considerable progress up the trunk, especially in the wood (cf. Plate XXXIV, fig. 3), and in these trees boring beetles had begun to penetrate the diseased tissues. The shot-hole borer usually attacks portions of rubber trees which have been previously injured by some fungus; the region attacked by these insects is often not affected by a fungus advancing from the root system but in the specimens now referred to there was clearly a connection between the attack of the borers and the advance of *Sphaerostilbe repens* into the trunk. As the writer has pointed out elsewhere (5), rubber trees affected by the fungus *Ustulina zonata* advancing upwards from the collar are sometimes attacked by borers in the same manner.

Most of the trees seen to be affected by *Sphaerostilbe repens* were magnificent specimens of plantation rubber, 15 to 20 years old; the other trees had been recently brought into the tapping round or were ready for tapping.

The fructifications of *Sphaerostilbe repens* are of two kinds, both being minute. The first form to appear is the conidial stage which consists of white or pinkish white blobs about the size of a pin's head borne at the ends of pink stalks which are about $\frac{1}{16}$ to $\frac{1}{8}$ inch long and are hairy when young (cf. Plate XXXIII, fig. 2). This is the *Stilbum* stage and it arises from portions of the host permeated by the fungus or directly from the rhizomorphs; the conidial fructifications have also been seen on clayey soil lying in contact with diseased roots. These reproductive bodies have occasionally been found below the surface of the ground. The spores which arise at the extremities of the *Stilbum* type of fructification are hyaline, oval, and $10\text{–}20\mu \times 5\text{–}9\mu$ in size. Another *Stilbum* is an exceedingly common saprophyte on dead portions of rubber trees. This is *Stilbum cinnabarinum*, the conidial stage of *Megalonectria pseudotrichia*, readily distinguishable from the conidial stage of *Sphaerostilbe repens* by its red colour and smaller size. After the formation of conidia the fungus sometimes produces small, dark red perithecia, but I found these only rarely.

Sphaerostilbe repens sometimes lives entirely as a saprophyte on dead plant tissues and doubt has been expressed whether it is really parasitic on the roots of *Hevea*. In rubber trees affected by it that I was able to examine it was undoubtedly advancing into living tissues and therefore acting as a parasite. The actual means by which infection of the roots is effected by this fungus are unknown. The roots may sometimes have been already injured by adverse conditions such as bad drainage, and if the fungus entered such roots it might easily pass thence into healthy tissues.

Pure cultures of the fungus are easily established by sowing conidia upon sterilised blocks of *Hevea* wood or upon potato agar. A white mycelium rapidly develops which assumes first a pink and then a brownish tinge. After a time, sessile aggregations of spores of a yellowish pink colour arise on both media and these are often arranged in concentric zones. These spores are hyaline, oval, and very variable in size, the average limits being $16–20\mu \times 6–8\mu$ though some are much smaller (cf. Plate XXXIV, fig. 4).

Spherical, thick-walled resting spores $9–10\mu$ in diameter are formed in the hyphae and at the ends of short branches; these spores are brown in colour when mature. On blocks of *Hevea* wood, stalked conidial fructifications often arise similar to those which occur naturally. The mycelium penetrates the middle of these wood blocks, being specially abundant in the vessels and medullary rays. There was no sign of the formation of perithecia in these cultures.

Roots of seedling rubber plants in pots and of 4 year old trees on hilly land were inoculated with pure culture material of the fungus. In some cases the roots were wounded, in others the wood block bearing the mycelium, or the culture on agar, was placed against uninjured tissues. After an interval of nearly five months none of these plants (16 in all) showed signs of infection. This negative result points to the possibility that some, at present unknown, condition which disposes to susceptibility must exist before the fungus can invade the roots of a rubber tree. As pointed out above, bad aeration of the soil consequent on deficient drainage may be a factor in inducing the requisite condition for the fungus to enter.

Rubber trees affected by this fungus should be cut out and burnt. The attack is usually far advanced before the tree is seen to be diseased, hence it is generally not worth while to try to save the tree by excision of the affected parts. There is yet no evidence that the fungus spreads by subterranean strands but neighbouring trees should be examined, and to be on the safe side a trench should be dug around the affected tree at such a distance as to preclude the possibility of underground infection. If it is intended to replant the affected area after an interval, the soil should be deeply dug, all timber removed, and a liberal dressing of lime applied. This disease spreads more slowly than *Fomes lignosus*, and though it is not abundant in Malaya it is probable that the loss of some old rubber trees attributed to *Fomes* was really caused by *Sphaerostilbe repens*.

5. *Hymenochaete noxia*, Berk.

As pointed out by Brooks and Sharples (8) the Brown Root disease of plantation rubber is not of frequent occurrence in Malaya where it is commoner on trees about one-and-a-half or two years old than on mature trees. Petch (10) states that it is probably the commonest root disease of rubber in Ceylon although it causes less damage than *Fomes lignosus*. It is specially prevalent there in old cacao land. Many Malayan estates on the other hand have never been troubled with a case of *Hymenochaete*. As is well known, roots affected by this fungus are invested with masses of soil and small stones which become cemented to the bark. Patches of brown mycelium are frequently found intermixed with the débris on the exterior of the root, hence the popular name of the disease. The fungus does not produce mycelial strands which travel through the soil, so infection must result either through the roots coming into contact with other material containing the fungus or directly from spores. The fructifications of this fungus have not yet been found in Malaya, and Petch (10) states that its fruit-bodies have only occasionally been seen in Ceylon.

Trees affected by *Hymenochaete noxia* should be burnt, and in order to act on the safe side the area around them should be isolated by a trench. If the ground is to be replanted it should be dug over, freed from timber, and well limed.

Although it is customary to refer the brown mycelium associated with this disease in Malaya to *Hymenochaete noxia*, Berk., I have not seen the fructifications of this fungus and therefore cannot confirm its identification.

6. *Ustulina zonata* (Lév.), Sacc.

It has been thought desirable to include in this paper a summary of my investigations upon the root disease of plantation rubber caused by *Ustulina zonata*, a full account of which has appeared elsewhere (5).

This disease had not previously been recorded on rubber in Malaya, but Petch (11) had recently noted its occurrence in Ceylon estates especially where *Hevea* had been planted amongst tea which had been subsequently allowed to die out. In Ceylon this fungus causes a serious root disease of tea.

In Malaya this disease by no means infrequently attacks old rubber trees, although it is only in the few estates where groups of trees have been killed that serious damage has yet been done by this fungus.

The part of the tree chiefly affected is the collar which is usually attacked first on one side only, the bark collapsing. Neighbouring lateral roots and the tap root often become affected in the same manner and in advanced cases the disease may spread up the trunk to a height of three or four feet. If the diseased tissues are exposed, conspicuous black lines are often seen near the limits of the affected parts although these lines are not invariably present. The absence of external mycelium and of rhizomorphic strands between the bark and the wood distinguishes this disease from other well-known root troubles of *Hevea.* As the fungus progresses in the collar and root system of the tree the foliage becomes thin, the branches die back, and the whole tree succumbs unless successfully treated.

The fructifications of the fungus appear as closely adpressed plates, grey brown to blackish in colour, on the collar and exposed lateral roots of affected trees. The fructifications are easily overlooked, especially in wet weather when they become splashed with mud. The conidial stage present in young specimens of the fungus in Ceylon has not yet been found in Malayan specimens although adult fruit bodies are undoubtedly identical. The mature plate-like fructifications which may be several inches across are marked by an irregular and obscurely zoned surface punctured by minute dots. If the grey brown surface of a fruit body is scratched, a black layer is seen which may also become exposed by the natural wearing away of the thin covering. Below this black layer the perithecia are formed from which the spores subsequently exude in black masses.

Wood and bark invaded by the fungus become discoloured. The black lines often found near the margin of the affected tissues are caused by the aggregation and darkening of the hyphae which form a kind of sclerotic plate in the cells of these regions. Pure cultures of the fungus were established on blocks of *Hevea* wood in which similar black lines were formed. It is only when black lines in the tissues are associated with an extensive affection of the collar and root system of the tree that *Ustulina zonata* should be suspected, as two other fungi, *Nummularia pithodes* (B. and Br.), Petch, and a species of *Xylaria* both belonging with *Ustulina zonata* to the Xylariaceae and both common saprophytes on dead rubber wood, produce similar black zones. Inoculations of the roots of seedling *Hevea* plants and of 4 year old trees, just below soil level, with material of the fungus growing in pure culture were followed by the establishment of the fungus in the tissues, some of the seedlings being killed in consequence.

In Malaya it is likely that the fungus often begins to grow on decayed stumps from which it passes to the rubber trees, the fungus spreading from one tree to another by contact of diseased roots with healthy ones. On some of the older estates in which the disease has been found, however, very few stumps remain and it is likely that there are other means of infection. Some of the old trees affected by *Ustulina zonata* had been previously attacked by white ants. White ants frequently invade rubber trees attacked by *Fomes lignosus* and the reverse process may possibly occur in the case of *Ustulina zonata.*

It is important that this disease should be dealt with at an early stage although it is then rather liable to be overlooked. If the condition of the bark on one side of the collar of the tree arouses suspicion it should be examined, and if found to be diseased all discoloured tissues should be cut out and burnt and the exposed surfaces tarred; diseased lateral roots should be destroyed unless they are large, when the unhealthy tissues should be excised. If the fungus has penetrated so far into the tree that it would fall if all the affected tissues were cut out, the tree is doomed, but as the fungus spreads only slowly the tree may be kept in tapping until it ceases to yield latex in paying quantities. In order to act on the safe side, infected areas should be isolated by means of trenches.

7. *Botryodiplodia theobromae,* Pat.

After *Corticium salmonicolor,* this fungus causes the greatest amount of injury to the shoot system of *Hevea* in Malaya. *Botryodiplodia theobromae* may enter the tree in several ways; it may invade young shoots killed by *Gleosporium albo-rubrum* or by *Phyllosticta ramicola,* it may enter branches already attacked by Pink Disease caused by *Corticium salmonicolor,* or it may act as a wound parasite without association with attack by any other fungus. Whatever the mode of entry, *Botryodiplodia theobromae* spreads rapidly downwards in the tissues killing the branches and main stem as it proceeds so that the disease is popularly called "die-back." The fungus usually advances faster in the wood than in the bark, though in some trees attacked during 1914 the reverse was apparently the case.

Petch (10), Richards (12), and Bancroft (1) all point out that *Botryodiplodia theobromae* often attacks groups of rubber trees simultaneously and the same tendency has been noted by the writer. Rubber stumps have been affected by the fungus soon after being planted out though it was not possible to determine the manner of infection.

The only mode of treatment is to cut out and burn all affected parts as soon as the disease is seen. Prompt action is particularly necessary in dealing with this disease on account of the rapidity with which it develops. If an estate is being thinned out, the rubber trees which are discarded should not be allowed to remain lying in the plantation indefinitely as such material offers a good breeding-ground for this fungus.

Many different names have been given to this fungus but *Botryodiplodia theobromae* has the right of priority as far as its common conidial stage is concerned. In 1911 Bancroft (1) described the development of an ascus stage in its life-history. During my residence in Malaya I devoted special attention to trying to find the perithecia described by Bancroft but without success. Branches of *Hevea* attacked by *Botryodiplodia* were kept under conditions favourable for the formation of perithecia but in no case did ascus formation result. As far as I am aware no other observer has found the *Thyridaria* which Bancroft considers is the complete stage of the *Botryodiplodia*, and until confirmation of the presence of a perithecial stage is obtained it seems preferable to retain the name *Botryodiplodia theobromae* for the fungus.

8. *Bark Diseases.*

Diseased bark was frequently seen in rubber trees, but apart from a careful search in vain for the bark canker of Ceylon attributed by Petch (10) to *Phytophthora Faberi* I had no time to investigate troubles of this nature in detail. As soon as the bark of a rubber tree becomes diseased, boring beetles are almost certain to attack the affected tissues. Where boring beetles attack a tree high up there is obviously no connection between their invasion and the action of a root parasite as described above under *Ustulina zonata* and *Sphaerostilbe repens*. I have occasionally seen borers penetrating the laticiferous layer of healthy bark by continued attacks, but usually they can only successfully invade bark which is diseased.

None of the diseased bark examined in Malaya presented the features associated by Petch (10) with the bark disease caused by *Phytophthora Faberi* in Ceylon. Many attempts were made by culture experiments to isolate a *Phytophthora* from bark taken from the junction of healthy and diseased tissues but in place of it some species of *Fusarium* or other hyphomycete was invariably obtained. This, however, is not sufficient evidence to justify the belief that species of *Fusarium* are capable of causing disease of *Hevea* bark. No mycologist who has resided in Malaya for any considerable period has yet isolated a *Phytophthora* from diseased rubber bark, hence the statement of Rutgers (15) of Java who after a brief visit to the Federated Malay States, and without isolating the supposed causative fungus, announced the presence there of a bark canker caused by *Phytophthora Faberi* cannot be considered conclusive. Until the fungus has been isolated from diseased bark and has successfully infected trees which have been inoculated it seems premature to say that the bark canker caused by *Phytophthora Faberi* occurs in Malaya.

Rutgers and Arens (16) consider that the burrs on rubber trees are often due to *Phytophthora Faberi*, a view for which the writer does not think sufficient evidence has yet been brought forward.

9. *Burrs.*

There are two kinds of burrs on rubber trees, one consisting of small, pea-like swellings in the bark, the other being irregular woody growths extremely variable in size which arise both on tapped and untapped surfaces though chiefly on the former. The pea-like nodules can be cut out with a knife as they are somewhat easily separated from the surrounding tissues. This kind of burr is of little economic importance. The irregularly shaped burrs on the other hand are of considerable economic importance as they seriously incommode tapping operations and may even render the renewed bark incapable of being tapped effectively. These burrs are specially abundant on trees that were overtapped during the boom of 1910.

Apart from Rutgers and Arens (16), mycologists are in agreement that burrs on rubber trees are not due to the action of parasitic organisms but are caused by some physiological disturbance.

Bateson (3) attributes the formation of the "pea" type of burr, which often occurs on the sites of old leaf scars, to the stimulus set up by the coagulation of latex in the tubes which formerly passed out to the leaves with the vascular bundles. Richards and Sutcliffe (13) put forward the view that the large, irregular burrs are likewise caused by the stimulus on the surrounding tissues set up by the coagulation of latex in tubes which belong to the laticiferous system of the stem. There is good evidence for both these views. A small percentage of burrs is doubtless due to wounds made in the wood through bad tapping.

With the conservative and more careful methods of tapping in general use at the present time, burrs will probably be less troublesome in the future than in the past. The larger type of burr is difficult to treat but good results appear to have been obtained on some estates by using a plane to make the surface smooth after removing these irregular excrescences.

10. *Thread Blight.*

A white thread blight is of common occurrence on rubber trees in Malaya but it causes little harm. The mycelial strands of this fungus vary considerably in size and run long distances over the branches, matting the finer twigs and leaves together and sometimes enveloping the leaves with a fine felt of hyphae causing them to die. The fungus is conspicuous and should be cut out before it has had time to do any considerable harm. This white thread blight is very variable in character and it is possible that more than one species of fungus is involved. Thread blights rarely fructify and there is at present only one record of a fruiting stage in Malaya observed by Richards (12) who sent the fungus to England for identification. It was named *Cyphella Heveae* by Massee and is thus a member of the Thelephoraceae. Thread blight is of common occurrence on other cultivated trees in Malaya.

11. *Phyllosticta ramicola*, Petch.

This fungus affects young green twigs of *Hevea brasiliensis* and sometimes affords opportunity for the entrance of *Botryodiplodia theobromae* as pointed out by Richards (12) and Bancroft (1). It occurs more frequently on trees one to two years of age than on older ones and is usually most abundant towards the end of the year when the rains are heaviest. The fungus generally enters the twig at one or more of the leaf axils 6 to 18 inches below the apex, the fungus probably beginning the attack in the leaf axils because moisture is retained here better than elsewhere. The first sign of its development is the appearance of small brown patches which spread upwards and downwards causing first a blackish and then a brownish discoloration of the stem. At the same time enormous numbers of pycnidia scarcely visible to the naked eye are formed on the discoloured parts. The disease spreads rapidly downwards and as much as 3 feet of the stem may be killed in the course of a week. The extremities of the affected twigs, which are often not invaded by the fungus, die in consequence of the failure of the water supply caused by the presence of the fungus below. On one low-lying part of an estate an outbreak of this disease was almost epidemic in character, more than 200 trees, one to two years old, growing on peaty soil being affected together. Trees of the same age on higher land with better soil remained unaffected. Poor drainage and a peaty soil often check the growth of rubber trees which appear generally to be more subject to disease under these conditions, possibly on account of a lessened resistance to parasitic organisms.

Branches affected by this fungus should be cut out and all diseased portions burnt. If an attack on young trees is observed in an early stage the trees which are still healthy should be sprayed at intervals with Bordeaux mixture.

A *Phyllosticta* was also sometimes found on the margins of rubber leaves and inoculations showed that it could act as a weak parasite causing a brown discoloration of the leaves from the margin inwards. The spores of this *Phyllosticta* appeared to be identical with those of *Phyllosticta ramicola* but it was not possible to carry out comparative cultures to test this.

12. *Gleosporium albo-rubrum*, Petch.

Petch (10) first described this fungus as causing a die-back of the green shoots of *Hevea* in Ceylon which was often the forerunner of attack of the woody parts by *Botryodiplodia theobromae.* The fungus plays the same rôle in Malaya and what is apparently the same fungus often affects young leaves also. Both upon the young stem and upon the leaves the pustules of this fungus are pink in colour, the individual spores being hyaline and 14–20μ × 4μ in size. On young stems of *Hevea* I have sometimes seen this fungus intimately mixed with *Phyllosticta ramicola.* Where *Gleosporium albo-rubrum* occurs on recently unfolded rubber leaves it causes them to shrivel from the margin and fall rapidly from the tree. On a few mature trees growing in low-lying land I have seen this fungus so abundant at the time of unfolding of the leaves after "wintering" that the ground below was thickly carpeted with the leaves which had been shed. Leaves of *Hevea brasiliensis* at the time of unfolding are of delicate texture and are much less resistant to fungoid attack than when fully developed. This was the only indication I saw of any present danger of a serious leaf parasite of plantation rubber.

13. *Cephaleuros* sp.

Although this alga is of no economic importance on rubber as is *Cephaleuros* on tea it is interesting to note that an organism of this nature can penetrate the leaves of *Hevea*, on which it is often found in the form of small brown spots more especially on the under-surface. Filaments of the alga grow out from these spots and terminate in groups of sporangia as shown in Plate XXXV, fig. 5. This alga is of common occurrence on the leaves of other plants in Malaya and is specially abundant on clove in which Ridley (14) states that it causes a serious disease.

14. *Loranthus* spp.

As pointed out by the writer (6) in a Malayan publication, two kinds of *Loranthus* occurred as semi-parasites on the branches of old rubber trees in a few estates in Negri Sembilan and in one or two areas some damage was being done by them. Bateson (4) has also recorded the presence of a species of *Loranthus* on rubber in Pahang. In the Negri Sembilan estates, portions of branches beyond the place of attachment of the parasites were often killed. The trees most severely affected were generally in poor condition, being badly burred and having been overtapped some years before. The foliage was thin and this circumstance probably assisted the parasites to become established, for in trees possessing a vigorous leaf canopy the shade cast by the crown tends to prevent the establishment of these troublesome plants. The development of *Loranthus* on a rubber tree is a drain upon its resources, and if such "mistletoes" are allowed to develop with impunity the tree will become impoverished, just as apple trees in some parts of England are weakened by the unchecked development of *Viscum album*.

All branches of rubber trees affected by these growths should be cut back well beyond the region to which the parasite extends.

Various species of *Loranthus* are of common occurrence in Malaya on many kinds of trees and shrubs. One of the species found on rubber trees grows frequently on *Melastoma malabathricum*, which is one of the commonest wayside shrubs in Malaya. The species of *Loranthus* seen on rubber trees are provided with runner-like processes which creep along the branches of the host giving off suckers here and there which form the means of attachment and the channels by which food substances are obtained. Some of the suckers become much swollen and the stem of the host in the immediate vicinity is abnormally developed, the whole producing a large knob of hypertrophied tissue. Other suckers which are much closer together than the former kind are not associated with any marked hypertrophy of the host.

The leaves of these species of *Loranthus* are extremely variable in size and shape. Plate XXXV, figs. 6 and 7 are photographs of one of the kinds of *Loranthus* which affects rubber trees showing the creeping stems and the hypertrophied tissue where the parasite penetrates the host deeply.

In conclusion I wish to express my hearty thanks to Mr F. de la Mare Norris of the Agricultural Department, Federated Malay States, for permission to reproduce his drawings of *Sphaerostilbe repens* in Plate XXXIII, figs. 1 and 2, and for making the drawings for Plate XXXIV, fig. 4 and Plate XXXV, fig. 5. I am also indebted to Mr A. Sharples of the same department for taking for me the photographs used in Plate XXXV, figs. 6 and 7.

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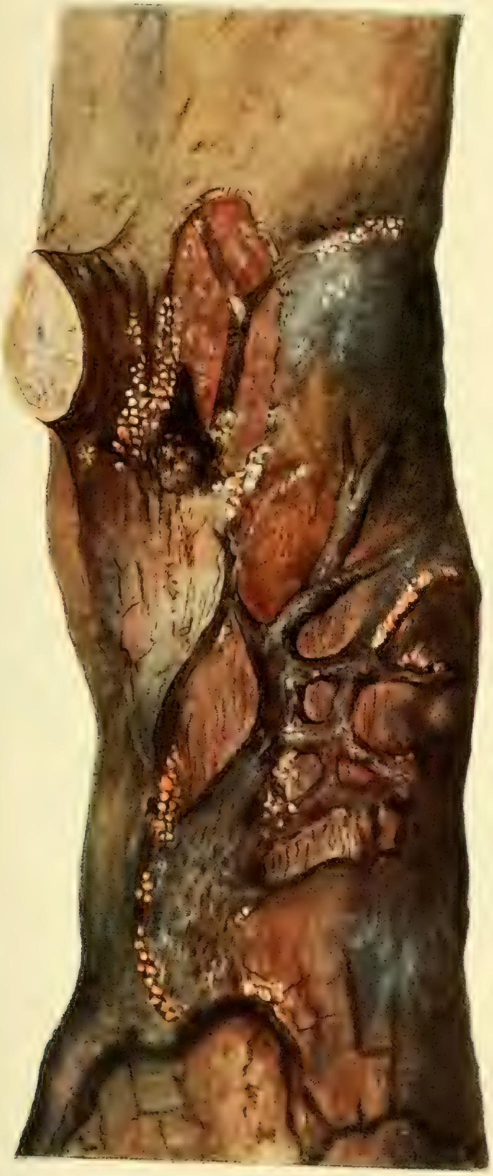
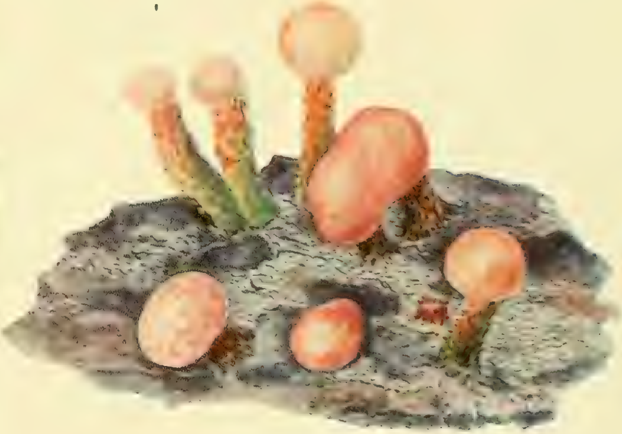


× 1.

Fig. 1



× 30 diam.

Fig. 2

Sphaerostilbe repens, B. and Br.

Diseases of Plantation Rubber

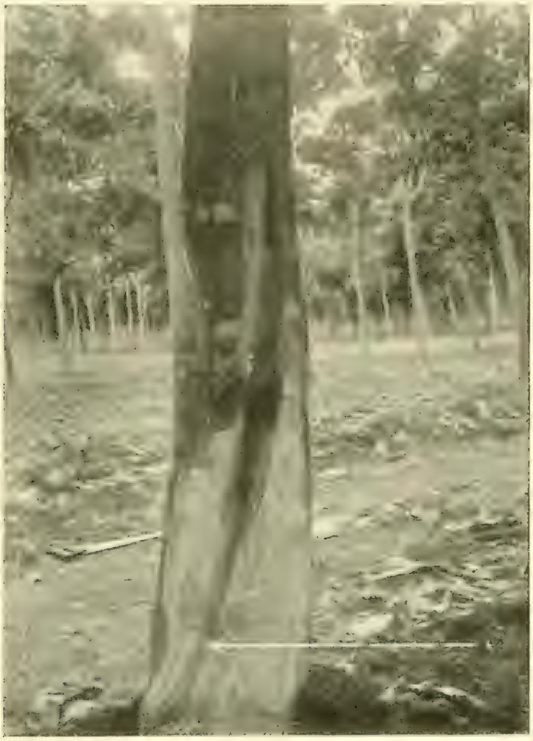


Fig. 3

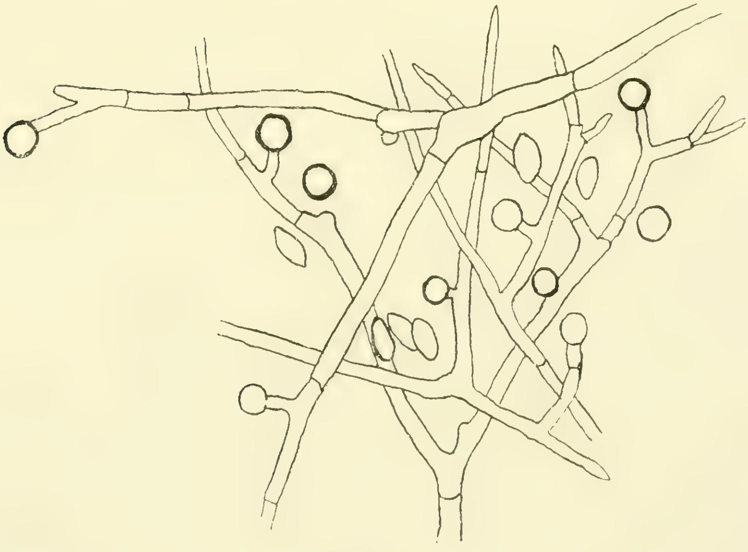


Fig. 4

Diseases of Plantation Rubber

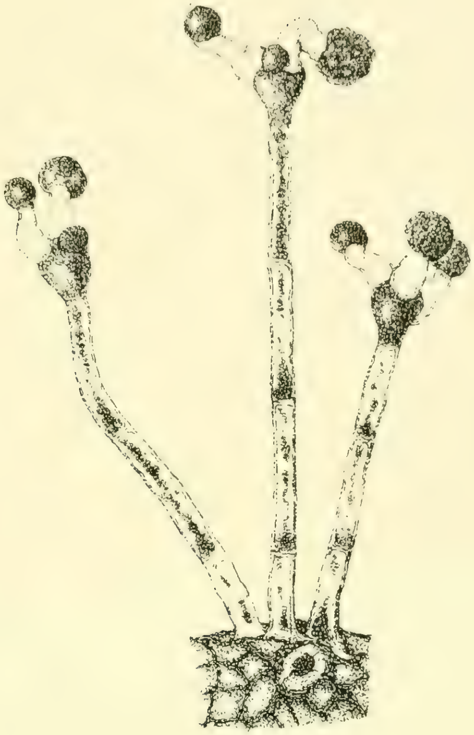


Fig. 5

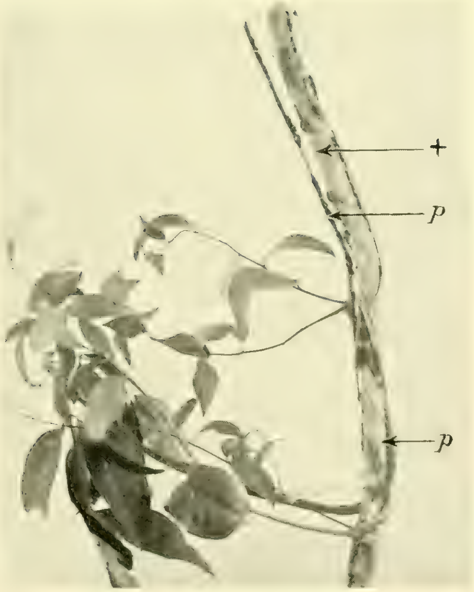


Fig. 6

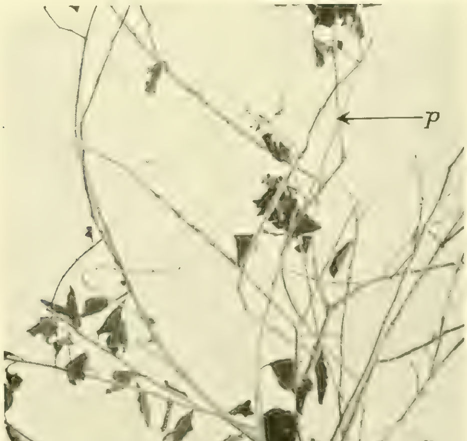


Fig. 7

Diseases of Plantation Rubber

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EXPLANATION OF PLATES.

PLATE XXXIII.

Fig. 1. Root of rubber tree with strands and conidial fructifications of *Sphaerostilbe repens*. Natural size. (Water-colour sketch by Mr F. de la Mare Norris.)

Fig. 2. *Stilbum* stage of *Sphaerostilbe repens*. ×30. (Water-colour sketch by Mr F. de la Mare Norris.)

PLATE XXXIV.

Fig. 3. Photograph of rubber tree attacked by *Sphaerostilbe repens* and by boring beetles. The bark has been removed to show the upward extension of diseased wood (*d*) from the root system.

Fig. 4. Conidia of *Sphaerostilbe repens* formed in a pure culture on *Hevea* wood. Some spores are thick-walled. ×450.

PLATE XXXV.

Fig. 5. *Cephaleuros* sp. Material growing on a clove leaf. The species on rubber leaves is apparently the same. ×600.

Figs. 6 and 7. Photographs of branches of rubber trees attacked by *Loranthus*. + is a branch of *Hevea*, *p* is a part of the parasite, *h* in Fig. 7 is a mass of hypertrophied tissue belonging chiefly to the host.

NOTE ON DR J. SMOLÁK'S PAPER "A CONTRIBUTION TO OUR KNOWLEDGE OF SILVER-LEAF DISEASE."

(*Annals of Applied Biology*, July, 1915.)

In reading Dr Smolák's interesting paper I gather that he does not fully understand my views on Silver-leaf disease. As his presentation of my views may lead to misunderstanding, I wish to take this opportunity of correcting any misapprehension that may arise.

On page 154 of his paper the following statement occurs: "According to the investigations of..... Brooks the basidiomycete *Stereum purpureum* is the cause of Silver-leaf disease as was shown by successful inoculation experiments," the investigations referred to being those described in the *Journal of Agricultural Science* for June, 1913. This statement does not agree with my own deductions from these investigations which were expressed in that paper in the following words:

> "In a previous paper (*Journal of Agricultural Science*, 1911) ....I pointed out it would be rash to say that *Stereum purpureum* was the *only* cause of Silver-leaf. Recent investigations have strengthened this view, for specimens of silvered foliage have been seen which I am unable to attribute to the action of *Stereum purpureum*" (page 288).
>
> "....silvering of foliage is a widespread phenomenon which is probably induced by various means, the chief one of which in the fruit-growing districts of this country being the fungus *Stereum purpureum*" (page 288).
>
> "It has been shown that Silver-leaf is a pathological condition of widespread distribution, the chief cause of the malady in the fruit plantations of this country being the fungus *Stereum purpureum*. ..... Examples of silvered foliage have, however, come under observation which, in my opinion, cannot be attributed to the action of *Stereum purpureum*. It is unlikely that the silvering of the leaves of seedling Plums and of such a plant as the White

> Dead Nettle is caused by this fungus. I look upon Silver-leaf as a general pathological phenomenon which may be caused in various ways, although at present only one of these agents, the fungus *Stereum purpureum*, is known with certainty" (page 306).

These extracts show a considerable divergence between Dr Smolák's statement quoted above and the views concerning Silver-leaf enunciated by me. The same opinions on the disease were also expressed by me in an article that appeared in the Journal of the Board of Agriculture for November 1913.

On page 155 of his paper Dr Smolák after quoting descriptions of two cases in which I pointed out there was no connection between silvering and *Stereum purpureum* or *vice versâ* says: "Surely in the light of this evidence it is not possible to believe that *Stereum purpureum* is the sole cause of the disease!" The above quotations from my paper show that this is the substance of the view expressed by me in 1913 though Dr Smolák makes no mention of this fact.

F. T. BROOKS.

CAMBRIDGE,
August 18*th*, 1915.

SPRAYING FOR APPLE SUCKER (*PSYLLA MALI*).

BY F. R. PETHERBRIDGE, M.A.

(*School of Agriculture, Cambridge University.*)

It is a common practice among fruitgrowers to spray their apple trees at the time when the buds are bursting, in order to check the ravages of the apple sucker. It is supposed that the washes applied prevent the eggs from hatching. Some experiments[1] were carried out in 1914 by the writer to try and find out how much good the various washes did in this connection.

The following experiments are a continuation of this work in more detail.

The experiments were carried out in one of Messrs Chivers and Sons' orchards. Each plot contained ten apple trees alternated with plum trees, and was separated from its neighbour by a row of plum trees. The trees were about twenty years old and all of the variety Keswick Codling.

At the end of February the number of apparently fertile apple sucker eggs were counted on certain twigs. A twig was chosen on each of three trees on every plot. The twigs were as similar as possible and were situated on the outside of the trees in order to prevent insects from falling from above. They were thickly covered with eggs, each carrying about 500.

A high pressure spraying machine was used for making the applications, and special care was taken to cover all the eggs which had been counted. It should be noted that on many of the trees, although carefully sprayed, about 20 % of the eggs were untouched by the spray. Observations were also made in the orchards of other growers, and it was found that a considerable proportion of the eggs are not covered by the spray fluids used.

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[1] *Journ. Board of Agric.* Jan. 1915.

In these experiments the average amount of fluid applied to each tree was about six gallons.

Two plots were used for each mixture. The first application was made early in March just as the buds showed signs of swelling, and the second application was made a fortnight later just as the leaves were beginning to show.

Plots *B, D, F, I, K,* and *M* were sprayed on March 4th and 5th in fine weather, and rain fell on March 6th.

Plots *C, E, G, J, L,* and *N* were sprayed on March 18th.

There was a little snow falling while these plots were being sprayed but not sufficient to prevent the sprays from drying on the trees. After the spraying there was a heavy fall of snow which washed off some of the spray.

After spraying a band of Tanglefoot was placed round each of the 42 twigs in order to prevent any young suckers from crawling on or off the shoots.

Twigs were cut on March 13th and kept in the Laboratory and on these the suckers began to hatch on March 20th. On the plots they did not begin to hatch until April 8th. This lateness of hatching was probably due to the cold nights experienced after the second spraying as the buds were sufficiently advanced to provide nourishment for the suckers on March 18th, and it was for this reason that the second spraying was done earlier than was originally intended.

Maximum and Minimum Temperatures taken on the Experimental Plots.

	Max.	Min.		Max.	Min.
March 15	50	40	March 26	44	29
16	53	36	27	43	25
17	49	32	28	44	21
18	50	40	29	41	22
19	41	24	30	46	22
20	43	26	31	48	27
21	53	30	April 5	59	28
22	47	25	6	56	30
23	55	41	7	55	43
24	60	34	8	55	34
25	60	38			

The suckers continued to hatch until April 28th. The number of suckers which hatched were counted on April 29th to May 6th.

The following table gives the treatment, the number of eggs on each twig and the numbers which hatched.

Plot	Treatment	Twig	Number of eggs on twig	Number hatched	Percentage hatched	Mean
A	Untreated	*A* 1	466	424	90	83 %
		A 2	443	320	72	
		A 3	482	416	86	
B	Sprayed on March 5, 1915, with: lime ... 200 lbs water ... 100 gals self-boiled	*B* 1	610	334	55	57 %
		B 2	461	271	59	
		B 3	344	197	57	
C	Sprayed on March 18, 1915 Mixture as *B*	*C* 1	493	273	55	62 %
		C 2	599	409	66	
		C 3	496	325	65	
D	Sprayed on March 5, 1915, with: lime ... 150 lbs salt ... 30 lbs water ... 100 gals self-boiled	*D* 1	511	122	24	27 %
		D 2	632	236	37	
		D 3	678	138	20	
E	Sprayed on March 18, 1915 Mixture as *D*	*E* 1	511	289	57	68 %
		E 2	467	407	87	
		E 3	442	262	59	
F	Sprayed on March 4, 1915, with: lime ... 40 lbs sulphur... 24 lbs water ... 100 gals boiled for 1 hour	*F* 1	468	437	93	76 %
		F 2	457	298	65	
		F 3	489	349	71	
G	Sprayed on March 18, 1915 Mixture as *F*	*G* 1	557	274	49	61 %
		G 2	516	347	67	
		G 3	338	222	66	
H	Untreated	*H* 1	562	404	72	88 %
		H 2	491	482	98	
		H 3	449	428	95	
I	Sprayed on March 4, 1915, with: commercial lime sulphur wash	*I* 1	465	408	88	87 %
		I 2	554	544	98	
		I 3	595	471	75	
J	Sprayed on March 18, 1915 Mixture as *I*	*J* 1	486	434	89	83 %
		J 2	498	311	62	
		J 3	502	488	97	
K	Sprayed on March 4, 1915, with: lime ... 40 lbs salt ... 12 lbs sulphur... 24 lbs water ... 100 gals boiled for 1 hour	*K* 1	424	411	97	84 %
		K 2	483	464	96	
		K 3	528	309	59	
L	Sprayed on March 18, 1915 Mixture as *K*	*L* 1	280	266	95	90 %
		L 2	472	452	95	
		L 3	643	522	81	
M	Sprayed on March 5, 1915, with: lime ... 12 lbs sulphur... 24 lbs water ... 100 gals boiled for 1 hour	*M* 1	507	431	85	82 %
		M 2	537	363	68	
		M 3	541	503	93	
N	Sprayed on March 18, 1915 Mixture as *M*	*N* 1	561	537	95	93 %
		N 2	492	475	96	
		N 3	449	402	89	

The above results although showing large variations for the same treatment give some idea as to the amount of suckers which are prevented from hatching by spraying at this time of the year. They indicate that the various kinds of lime-sulphur and lime-salt and sulphur are not effective in keeping down apple sucker; also that lime wash is partially effective and lime and salt wash still more effective. It is difficult to explain why the lime-salt wash which was applied on March 5th (Plot *D*) should be more effective than that applied on March 18th (Plot *E*) unless the heavy fall of snow which followed the spraying washed off some of the deposit.

Last year lime-salt and sulphur proved more effective than lime or lime and salt. The apple suckers which hatched out were so numerous that they seriously affected the crop on all the plots, and judging from the whole plots no very marked benefit as indicated by the above figures could be seen on Plot *D*.

It is possible that more good might have been done by a later application, but the buds were shooting when Plots *C, E, G, J, L,* and *N* were sprayed, and a later application last season injured the young foliage.

In the Active Season.

In this experiment most of the ordinary washes used against apple sucker were tried. The plots were similar to those in the above experiments.

The sprays were applied on May 7th, 1915, and at this time the flower trusses were opened, but the flowers themselves had not opened.

Plot *O*[1].	Soft soap	10 lbs
	Creosote oil (crude commercial)...	1 quart
	Water	100 gallons.

This wash was effective in killing the suckers but caused scorching of the leaves and cannot be recommended for Keswick Codlins.

Plot *P*.	Soft soap	10 lbs
	Nicotine (98 %)	8 ozs
	Water	100 gallons.

This wash penetrated well and was very effective in killing the suckers.

Plot *Q*.	Agricultural treacle	6 lbs
	Nicotine	8 ozs
	Water	100 gallons.

This wash penetrated well and was very effective in killing the suckers.

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[1] Recommended by Awati, *Ann. App. Biol.* Vol. I, 1914–15.

Plot *R*.	Soft soap	10 lbs
	Paraffin	2 gallons
	Water	100 gallons.

Almost as effective as *Q*.

Plot *S*.	Soft soap	10 lbs
	Quassia	10 lbs
	Water	100 gallons.

Almost as effective as *R*.

Plot *T*.	Soft soap	10 lbs
	Water	100 gallons.

Not so many suckers killed.

In this experiment the suckers were so numerous that these sprayings had no very marked effect on the yield. Most of the trusses fell off as a result of the injury done to them by the suckers in the young stages. This shows the necessity for preventing the suckers from hatching.

CONCLUSIONS.

These experiments indicate that lime and salt may be effective in preventing a large proportion of apple sucker eggs from hatching. Lime wash was also fairly effective.

Soft soap and nicotine, or treacle and nicotine, were the most effective after the suckers had hatched.

Spraying to prevent the eggs from hatching is not sufficient to keep this pest under control, but should be followed by an application of nicotine and soft soap, or treacle and nicotine, to kill those which have hatched.

My thanks are due to Messrs Chivers and Sons for their kind permission to use their orchard, and for bearing the expenses of the experiments.

ON THE BIOLOGY AND ECONOMIC SIGNIFICANCE OF *TIPULA PALUDOSA.*

By JOHN RENNIE, D.Sc., F.R.S.E.

(*North of Scotland College of Agriculture.*)

PART I. MATING AND OVIPOSITION.

(With Plate XXXVI.)

IN the course of the summers of 1913 and 1914 the mating and oviposition of *Tipula paludosa* formed the subject of special study both in the field and in laboratory experiments. As far as the writer knows no account of the sexual behaviour of this species is on record, at all events in detail. The points of special interest which have been made out are summarised in the present paper; the observations of the first season have been confirmed in the second.

Mating.

The earliest occasion in a season upon which mating was observed in progress was 20th June, 1914. This was in a field cage in which the flies had hatched out naturally. In the previous year, when a closer watch was kept, and the flies were reared in captivity and probably hatched earlier than in the field, the date was actually later, viz. 4th July. It may here be mentioned that, as detailed in a subsequent paper, there is only one generation of this species in the year in the north of Scotland. In the south of Britain it is held that two generations occur.

The flies experimented with were reared from larvae which had been kept in field and laboratory cages. The pupae were collected and transferred to small vessels about the size of ordinary flower pots

The work recorded in this and subsequent papers has been carried out with the aid of grants from the Board of Agriculture for Scotland.

containing soil covered with small pieces of turf. On the top of the turf was placed a glass cylinder about 9 inches high and 3½ inches in diameter. In some instances, lamp chimneys with nearly twice this width at their base were used. These were about 12 inches high but narrowed to about 2 inches diameter at the top. The vessel was kept closed by a piece of light cardboard placed upon the opening at the top. There were never more than two or three adult insects confined in these simple cages; they appeared quite comfortable and lived in most instances about a week, in some cases as long as eleven days.

The following observations are typical of the general results obtained and will serve as descriptive of the mating habits of these insects under the conditions which have been specified.

On the 5th of July, 1913 at 11.15 a.m. a pair of crane flies of the species *T. paludosa,* here referred to as *M* 1 and *F* 1, was found *in coitu* in one of the larvae rearing cages. They were removed without being separated to one of the glass observation vessels and watched until 1 p.m. at which time they still remained united. At 1.45 p.m. they were found to have separated. They were the sole occupants of this particular cage. This is referred to as Mating (*M* 1 + *F* 1)1.

At 2.30 p.m. on the same day they again spontaneously effected coitus, remaining attached until 3.30 p.m. Mating (*M* 1 + *F* 1)2.

At 4.15 p.m. on the same day mating was again accomplished between this pair. Coitus lasted until 5.23 p.m. Mating (*M* 1 + *F* 1)3.

The same evening these flies were placed together upon a small enclosed grass plot. For a short time they remained indifferent to each other, and the female commenced oviposition.

A second female, *F* 2, newly hatched, was placed beside them and almost immediately the male flew towards her and, after a short struggle in which some of the legs of the female were broken off, coitus was effected. Mating (*M* 1 + *F* 2)1.

At 10.20 a.m. on the following day a newly hatched male, *M* 2, was introduced to the vessel beside *F* 1, who had as already noted been ovipositing. Union was immediately effected. At 12 noon they still remained *in coitu,* and at this hour they were taken apart and placed in different vessels. Mating (*M* 2 + *F* 1)1.

At 12.15 p.m. *M* 1 was once more placed beside *F* 1. At 2.40 they had taken no notice of each other. They were now disturbed and made to fly about the cage. Upon coming into proximity they mated. Mating (*M* 1 + *F* 1)4.

At 4.30 they were found to have separated. They were left together overnight.

On the morning of the 7th, this female *F* 1 was removed to another vessel beside a male, *M* 5, hatched overnight. He flew at her instantly on her arrival and in a few seconds mating was effected, 9.55 a.m. At 11.20 a.m. they were found to have separated. Mating (*M* 5 + *F* 1) 1.

F 1 was now placed in a small glass vessel over some moist cotton wool. Up till 6.35 oviposition had not taken place under these conditions. At this hour she was placed over soil to see if this would now supply the stimulus. The result was negative. This female, however, was known to have oviposited previously, and at this stage she had lost the full-bodied appearance of the unmated female and was quite slender in form.

On the 9th, *F* 1 was placed with another male, *M* 6, but no union took place. From this date she was kept in the company of a male until she died. This occurred upon the 16th and meantime no further mating had been effected in her case. Reviewing the facts regarding her, it is noted that between the morning of the 5th and the morning of the 7th she mated at least six times, and with three different males. She lived 11 days in captivity.

The male *M* 1, as has already been recorded in the account of the behaviour of *F* 1, mated with her four times in the course of two days. He also mated with another, *F* 2, within a short time of her hatching. This took place at 7.10 p.m. on the 6th July. At 9.15 they were still mated, and on the following morning they were again found *in coitu*.

At 10.35 upon the morning of the 6th this male was introduced to a vessel in which there were already three females and one male. He at once flew amongst these and in a few seconds became coupled to a hitherto unmated female *F* 3. They remained *in coitu* until 12.15 when they separated. Subsequently as recorded for the female, *F* 1, he paired with her at 2.40 p.m. of the same day.

No further opportunities for mating were given to this male until the 9th (four days after he was first found mated), on which day he was placed with a newly hatched female *F* 9 at 11 a.m. They were left together until 3.20 p.m. but coitus did not take place. He mated at least seven times in the course of two days. He died upon the 12th, having lived seven days.

A number of similar experiments were performed and always with consistent results. Recently hatched insects paired most readily, and most were ready upon opportunity to pair a number of times. The following are additional notes.

F 12 placed in vessel with males, *M* 11, *M* 12, and *M* 13. *M* 11 attempted coitus and failed. *M* 12 succeeded. *M* 12 subsequently mated with *F* 13.

F 16, *F* 17, *F* 18 placed in a vessel containing a crowd of males. The males immediately flew to them and in a few seconds all three females were mated.

F 20 and *M* 17, both hatched on the morning of 18th July. They mated at 10.30 a.m. During the day she oviposited. *F* 21 and *M* 18, hatched later in the day, both mated. *F* 21 had defective wings. These four insects were placed together. Both pairs mated again in the evening. On the following evening *F* 20 mated once more with either *M* 17 or *M* 18. By this time she had become quite slender, and had evidently oviposited freely.

F 25, hatched on 28th July. Placed with *M* 24. Mated and oviposited. On the 30th she was placed with 14 males captured in the open. There was much commotion on her arrival and in a short time one of the males succeeded in mating with her.

F 27, hatched 6th August. Dropped into vessel containing *M* 24, *M* 25, and others. An immediate contest for her took place; mating successful.

F 28. Placed with the foregoing, with same result. The males immediately surrounded her, and mating was effected in the course of a few seconds.

In seeking to effect coitus, the male alights above the female and prevents her escape, should she attempt it, by intermingling of their limbs. Meantime his abdomen, which is now markedly upturned at the tip, is passed below that of his mate. The widely gaping claspers seize her on the thickened basal part of the ovipositor and the hold having been made secure the pair rest a few moments in this position. The male, now releasing his hold by the limbs, turns round so as to face in the opposite direction from the female. This is the position maintained until separation takes place. Sometimes the wings are folded, but more usually in both sexes they are extended. During coitus the antennae of the male continue in active backward and forward quivering motion. In his case too, the halteres quiver periodically in spasms of about a second's duration and at frequent but irregular intervals. This last feature was constantly to be observed and appeared to be directly associated with the sexual activity in progress. After attachment contractile movements are observable at the tips of both abdomens for a short period; subsequently the female rests absolutely passive. Cf. Plate XXXVI.

Oviposition.

This may take place quite early after mating. *F* 1 after having mated three times upon the 5th July commenced oviposition in the evening. *F* 2 mated on the 5th was watched in the process of egg laying on the morning of the 6th. Another female, *F* 5, hatched at 9.50 a.m. upon the 7th and mated immediately afterwards. She mated again with the same male *M* 12 in the afternoon. They separated at 4.20 and at 5.27 she was watched engaged in oviposition, and her eggs were collected when she had moved away.

Curtis (*Farm Insects,* p. 445) says: "the eggs are laid by the females as they fly or when they rest among the herbage and are propelled as from a pop-gun." So far I have not been able to witness this propulsion of the eggs while the insects were on the wing, but it will be understood that the conditions of my earlier experiments were not favourable to this method.

I have repeatedly watched the process amongst grasses. The female stands in a vertical position with the ovipositor pushed well down and into the soil if she can reach it. Sometimes a backward and forward screwing motion of the body is indulged in so that the ovipositor is bored well into the ground. Spasmodic jerks of the hinder part of the abdomen indicate the expulsion of the eggs one by one. After a few minutes she moves along a little way and the process is repeated. About half-a-dozen eggs may be deposited at the same spot, frequently fewer. In one case I was able on lifting a female, not actually ovipositing but exhibiting spasmodic movements of the ovipositor, to get her to lay an egg upon a card in my hand.

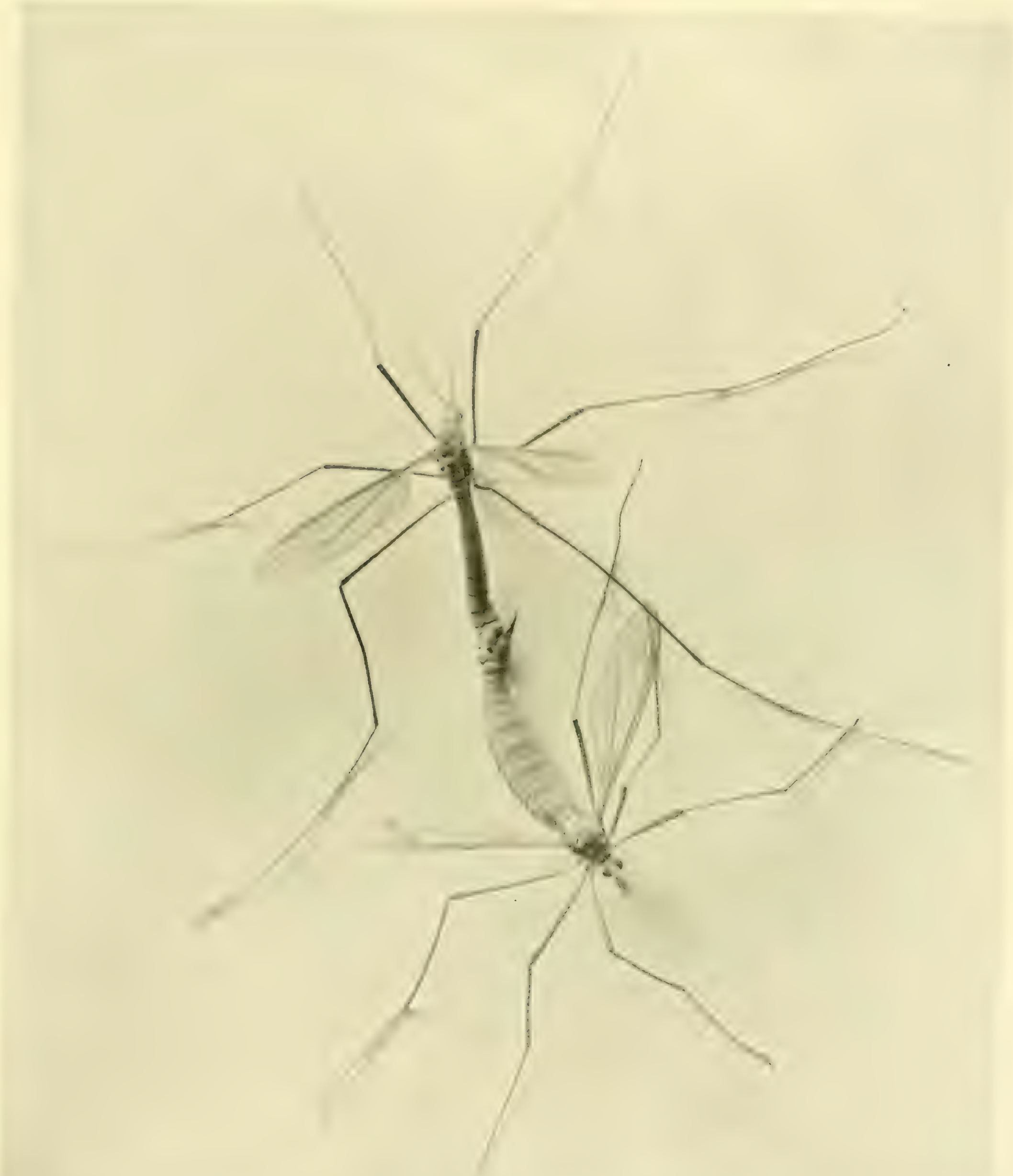
Newly hatched females are bulky in appearance at the anterior end of the abdomen. After oviposition they become slender. In this way one can tell whether a female has oviposited or not, though not generally whether the process is completed or not. The eggs in the newly hatched female mostly show black in colour through the skin and are shelled before fertilization. Advanced female pupae have the abdomen filled with the ovaries which are of a pale salmon pink colour. Dissection of females some time after oviposition shows that all the ova are not mature at hatching but that there is at least a second batch of eggs. Females captured in the open which are slender bodied, *i.e.* which show signs of having oviposited, have small pear-shaped ovaries occupying the posterior part of the abdominal cavity. The eggs in these may be well-developed and of the typical shape as when mature, but of the pale salmon colour. This suggests that females having oviposited may continue to live and to produce a fresh race of ova. This second lot of ova is not merely mature at hatching but without shells: they increase in size during the adult period. A female, *F* 22, hatched on the evening of the 20th July, and which had been mated and had oviposited, died on the 26th. Dissection showed that all the black shelled ova had been laid, and that the ovaries were small in size and confined to the hinder part of the abdomen. In this case, however, the individual ova were much smaller and had much less yolk than was found in the fly captured in the open and which was therefore presumably older. The question of the length of life of individual crane flies has not so far been settled.

Experiments were performed to test the degree of stimulus needed for the act of oviposition. Flies which had been mated and were placed upon cotton wool did not oviposit. Also when the wool was placed upon a layer of soil, they still failed to respond. Only in a few cases were eggs laid upon bare soil, whilst amongst herbage they were deposited readily. Although crane flies are known to oviposit usually in grass, it was found that they may do so in standing corn also.

The flies kept in captivity were not fed. They usually had access to growing grasses, and to soil, both of which were watered. They were observed licking at moist soil and at the wet sides of the glass vessels in which they were confined.

EXPLANATION OF PLATE XXXVI.

View of *Tipula paludosa* in coitu. The halteres are not visible owing to their vibratory movements.



Mating of *Tipula paludosa*

SOME EAST AFRICAN INSECTS OF ECONOMIC IMPORTANCE.

By R. H. DEAKIN, M.Sc.,

Lately Assistant Entomologist, British East Africa.

THE economic entomologist in East Africa has many duties to attend to, and the time he devotes to research on insect life-histories (his real object) is often very interrupted. The following notes, being some of the results of intermittent research, during one-and-a-half years residence in the E. A. P., without arriving at any important conclusions, do at least, I think, throw some new light on the problems concerned.

LOCUSTS. Little attention in the past has been paid to locusts as it is only at long intervals that they have caused damage. Natives of various tribes speak of bygone years when locusts were very destructive, but native memory for dates is rather uncertain. During the first five months of 1914 unusual swarms of the adult locust, *Schistocerca peregrina,* and in places, hoppers, appeared throughout the Protectorate, not only in the areas of low rainfall of the N. and S.E. but in the better watered regions around Nairobi, Naivasha, Nakura, the Uasin Gishu and also near Lake Victoria. The damage done was small, only the eating of the tops of a few coffee bushes and some mealies, etc., being recorded: there appeared to be everywhere a sufficient natural supply of food. I was unable, during a journey which I undertook for the purpose, to obtain evidence showing that these locusts had oviposited in any of the areas mentioned. I concluded that they must have proceeded to more arid steppe-like regions for this purpose. Rainfall statistics threw no light on a belief which I entertained that this was an exceptional year of drought in the home of the locusts—wherever this may be. Some facts relating to the life-history of this locust have been worked out in German East Africa, but the questions "When will they come?" and "Why do they come?" are yet to be definitely ascertained. Nor is this a question of secondary importance to the Protectorate, in view of the fact that there is an ever-increasing area of the country under valuable crops.

PESTS ATTACKING COFFEE. One of the greatest insect enemies which the coffee-grower in the E. A. P. will have to face in the future is *Antestia variegata*. German East African authorities believe that this extremely active plant-bug can be combated with contact sprays. My investigations were tried in another direction and with some success. Where the occurrence of the insect has been noticed by planters from year to year, it has been found that its activity grows and wanes successively. Quite accidentally I discovered that the increase in number of the bugs is very largely governed by a Chalcid egg parasite. My investigations were interrupted by the war, but were sufficient to prove the importance of this control. The groups of Antestia eggs are laid both on the leaves of the coffee bushes and also very largely on the dead leaves, twigs, etc., which accumulate under the bushes. One Chalcid egg is laid in each Antestia egg and the degree of parasitism is often greater than 50 % of the total number of eggs. The shells of eggs which have been parasitized are easily recognized by their black appearance and by the jagged hole by which the parasite emerged. The Antestia eggs which are laid on the dead leaves below are freer from parasites, possibly because they are more difficult to find by the Chalcids. In the plantation in which my investigation was carried out the eggs laid actually on the coffee bushes were parasitized almost to extinction. I suggest it might be possible to collect the dead leaves, etc., beneath the bushes, to place them in receptacles and, whilst allowing the parasites to escape, to prevent the young bugs from leaving by means of a smear of castor oil and resin tanglefoot. The damage caused by Antestia to coffee trees, by piercing the unripe berries and especially by the production of adventitious shoots, through the killing back of the apical buds, thus complicating pruning to a fearful extent, may be very severe. When the parasite occurs, efforts should be made, by its study, to increase its intensity of action, and where it does not occur to introduce it. The parasite has been sent to Mr G. A. K. Marshall of the British Museum for identification.

SCALE INSECTS ON COFFEE. *Lecanium* (*Saissetia*) *nigrum*, Niet., was found at an altitude of 6000 ft. Many individuals were parasitized.

I have examined specimens of the common scale on coffee from many parts of the Protectorate, but have found no specimens of *Lecanium viride*—all proving to be the closely related *L. africanum*.

CUT WORMS AND COFFEE. The common cut worm is *Euxoa segetum*.

If the total loss to the planters, caused by this insect attacking young coffee, could be assessed, I think the result would be startling. The ring-barked tree may not die till months later, hence there is an additional loss of time. Many people believe in the growing of maize between the coffee and even in leaving weeds as food for the caterpillars; they forget that by so doing they are encouraging the moths to lay eggs in their plantations. The remedy lies in clean cultivation and the intelligent use of poison bait. As soon as the value of these methods is emphasized by those in authority the loss will be considerably reduced.

PESTS ATTACKING CITRUS. Citriculture should be an important and remunerative occupation in B.E.A. in the future and the importance of insects in this connection must not be forgotten.

Argyroploce leucotreta, Meyr. This is also a pest in S. Rhodesia. It causes extensive damage to the orange crop at the experimental farm at Kabete, B.E.A. The caterpillar, which feeds inside and ruins partly ripe oranges, pupates just beneath the surface of the soil. The eggs are small, flat and scale-like, and are, I believe, laid on the fruit. Experimental spraying of the unripe fruit with lead chromate was not a success, sprayed trees suffering equally with the controls. This was found to be because the minute caterpillar on boring through the surface of the fruit appears to be in a great hurry and merely bites its way below the surface, disgorging the tissue removed—it cannot therefore be poisoned at this stage. My study of this interesting pest was interrupted, but I found in the one or two cases which I examined that the newly hatched caterpillar, before entering the orange (no particular place being chosen), spent some time in the calyx cavity, at the top of the fruit. Does it feed there, and could it be poisoned if spraying were carried out before the calyx cavity becomes closed? (cf. *Carpocapsa pomonella*).

A small, unidentified red mite causes a scabbing and silvering of oranges; the leaves of the tree appear to be unaffected.

Although *Icerya purchasi*, the Australian bug, occurs in the Protectorate, it does not seem to rank as a pest. It did not appear to thrive on trees which were artificially infected with it, at an altitude of 6000 ft. As showing how healthy crops and trees may suddenly suffer from migratory swarms of insects in Africa, the following case is of interest. A large swarm of small leaf-eating beetles (Halticinae?) suddenly appeared in a citrus orchard where they remained some ten days and did considerable damage to the young foliage. They appeared immune to smoke and contact sprays and were difficult to catch in greasy bags on the ends of sticks. They finally disappeared completely after the trees had been sprayed with a stomach poison.

The rare butterfly, *Papilio mackinnoni*, was unexpectedly hatched out from caterpillars feeding on orange foliage.

Eriosoma lanigera on Apples. This pest thrives in the Protectorate, where there are one or two old apple orchards, which are not of blight-proof stock. The aphids penetrate to the cores of the apples. No winged forms have been seen.

Orgyia vetusta. The caterpillars of this moth are very harmful to the foliage of the quince, but the brood which was studied was almost exterminated by an Ichneumon parasite. The caterpillars always feed on the shady undersides of the leaves, and if violently shaken from the tree during the heat of the day they die upon the hot surface of the soil.

Duomitus capensis. The caterpillars of this Cossid moth bore into, riddle and kill the branches of the native tree *Cassia didimobotrya*.

Pests attacking Black Wattle (*Acacia decurrens*). A new pest of which I have never obtained the adult is the larva of what appears to be a Buprestid beetle. The eggs are laid in small scale-like cases on the surface of the trunk and branches; they are the only indication of the presence of the insect, and the cause of the tree's death is thus obscured. The flattened larvae bore in the cambium and, when numerous enough, kill the tree. I found both healthy and unhealthy trees to be attacked. The felling and bark-stripping of attacked trees and the removal of all weakly ones seem the best method of control. Trees might be girdled and, thus weakened, used as traps for the beetles to lay their eggs on.

Two allied pests in the form of a Jassid and a Capsid bug are very injurious to black wattle, large areas of which they attack. Nothing is known of their life-histories or natural food plants. It is not known whether they ever leave the wattle, on the sap of which they feed. Instead of the trees growing up straight and quickly, they come to a standstill and assume a miserable, entangled appearance, due to the production of numerous adventitious shoots. Often the tree grows away from this condition, but always is its growth delayed, a serious matter for the wattle-planter. In the chief wattle-growing area of B.E.A. the pests do not occur above an altitude of 6500 ft. and the chief estates escape. Slow initial growth caused by weeds, drought, weak seeds, bad planting and cultivation appears to aid these very serious pests.

WINTER COVER WASHES (*conclusion*).

By A. H. LEES, M.A.,

Plant Pathologist, University of Bristol, Agricultural and Horticultural Research Station.

At the end of the season 1913–14 the most satisfactory mixture found for covering and adhesive properties was one containing whiting, starch, glue and potassium dichromate. It had, however, two distinct disadvantages, first that hot water was required and second that its cost was too high. Accordingly in the season 1914–15 fresh attempts were made to find a mixture that did not labour under these disadvantages.

So far all substances that had been added to lime to increase the adherence of the mixture had had the opposite effect. Either they had caused brittleness or softness. What was clearly wanted was some body that would serve to tie the lime particles together without itself becoming crystalline or gritty when dry. In other words some suitable colloid substance seemed the most hopeful body to search for. Of these glue, starch, casein, resin and humus bodies immediately suggested themselves. It was clear from the beginning that only substances sufficiently cheap need be tried, as comparatively large quantities would have to be used in practice.

Glue had already been ruled out owing to its being rendered insoluble by lime but the others were given a trial. Starch mixes well with lime. The heat of the slaking lime can be used to gell the starch so that no hot water is required. It causes considerable thickening of the mixture and gives a hard and brittle coat. Table I (4).

Subjected to rain outside it becomes gelatinous and is soon washed off. It is therefore unsuitable. Resin was next tried. This could be incorporated in two ways. It could be added to the lime during slaking, in which case it did not increase the adhesiveness of the coat though neither did it detract therefrom, or it could be first boiled with caustic soda and the solution added. This gave a much softer coat than the control. Resin therefore was not tried further. Table I (2).

Casein was added during slaking of the lime. It is soluble in alkalies so that a good mixture could be obtained. The resulting coat was, however, soft and useless. Table I (3).

Dried dung was used as a source of humus bodies and was added either immediately to the slaking lime or after a preliminary maceration with caustic soda. In both cases the mixture was unsatisfactory. Table I (5).

The use of dung suggested the possibility of increasing adhesiveness by incorporating some fibrous substance in the same manner that hair is used in plasters for house walls. At first filter-paper was used and afterwards newspaper. In both cases the paper was first treated with caustic soda and macerated and then the pulp was added to the slaked lime.

A considerable number of mixtures of different strengths both with and without the addition of starch were tried. On the whole the fibre decidedly improved the coat as it did away with the tendency to flakiness, though the addition of caustic soda seemed always to increase the softness. The method therefore of washing the pulp free from caustic soda was tried and the resulting coat was certainly harder. Some of these mixtures (Table I (6)–(12)) were tried outside (Table II), but the results were disappointing as all were washed off in a comparatively short time if subjected to heavy rain, and none of them could be considered satisfactory. This being the case it is not necessary to enter into details of their manufacture.

The last mixture of Tables I and II containing boiled linseed oil might have given good results if it had been possible, economically, to have used larger quantities. Its expense, however, ruled it out.

Effect of alkalies on glue solution.

The statement is made in the text-books that gelatine, the essential body in glue, is insoluble in alkalies and that gelatine or glue should not be used in spray fluids where free alkali is present.

This fact explains why mixtures of lime and glue were utter failures. Not only have they no sticking power but the mixture gives a very thin coat, very much thinner in fact than the same quantity of lime without glue would have given. No doubt caustic lime has an energetic action on glue. The mixture gives off bubbles of gas indicating that the glue not only becomes insoluble but is decomposed at the same time. The thinning effect is very characteristic and may be explained hypothetically as follows:

When lime is slaked under water it breaks up into a great number of extremely fine particles. Whether it is the fineness of the particles or whether it is associated with some other property the solid matter appears to be in a semi-gelatinous state. It is bulky and only sinks very slowly. If allowed to dry and then re-wetted the semi-gelatinous state is not re-acquired, but the particles remain gritty as though they had become flocculated. If a solution of glue is added to milk of lime freshly slaked the probability is that the glue precipitate so formed unites the minute lime particles into larger ones, so that the mixture at once loses covering power and shows the characteristic "thinning." It seemed therefore profitable in view of the uncertainty of the action of alkalies on glue to investigate the reaction. When 10 % solutions of caustic soda were added to hot glue solutions an immediate fibrous precipitate was obtained. A similar result was obtained by the addition of a hot 1 % solution and an immediate slight turbidity with so weak an alkali as lime water provided hot solutions were used.

On the other hand if cold 10 % caustic soda solution was added to cold glue solution no precipitate appeared for several hours. The same, as was to be expected, happened if 1 % caustic soda or lime water were added in the cold. The fact that the reaction between alkali and glue could be slowed down opened up new possibilities for lime mixtures.

Further experimentation with milk of lime showed that thinning occurred if incompletely slaked cold milk of lime or completely slaked hot milk of lime were added to glue, while it did not occur if cold well-slaked lime were used.

In working with milk of lime it is of course impossible to see whether the gelatine of the glue is actually precipitated, but it is safe to assume that loss of covering power in the mixture indicates precipitation. From these facts it appeared probable that a satisfactory mixture could be made by adding glue solution to cold well-slaked lime. Such mixtures were made up in the laboratory and it was found that one having the quantities lime 20, glue 2, water 100 gave a firm but thin coat. No sudden loss of covering power was here noticed such as follows when hot or unslaked lime acts on glue, but the coat was rather thinner though much firmer than the control lime 20, water 100.

This slight loss of covering power is no doubt due to the glue in solution. Gelatine is used in some summer sprays in order to increase their wetting and spreading power and probably the same spreading effect took place in this mixture. By increasing the lime from 20 to 30 a thick and firm coat was obtained. This 30, 2, 100 mixture was tried out of doors where it resisted rain fairly well but still did weather somewhat. It appeared therefore that the glue was not made insoluble on the tree quickly enough. Two methods suggested themselves as likely to overcome the difficulty. The first was to use the lime warm and so to get the glue gradually to become insoluble as it dried on the tree.

This was actually done in one or two cases with successful results but it was found difficult to judge the correct temperature and success was always a matter of luck. Any delay in getting the spray on the tree, owing for instance to choked nozzles, resulted in the glue being made insoluble before it reached the tree and a loss of covering and adhesive power.

The second method was to use potassium dichromate as had already been done the season before when working with glue mixtures. This causes the glue to become insoluble when exposed to light, thus holding the lime coat together. This method proved quite successful. It is necessary, however, to use the correct amount of dichromate. If too much is used immediate thinning is produced and the mixture is spoiled. If only a little too much is used the lime coat on the tree becomes too flaky though at the same time remarkably hard. So hard is it that if one rubs one's hand on the trunk of a tree so sprayed no lime comes off at all and the surface gives one the impression of fine emery paper. The best quantity to use is expressed in the formula:

Lime	30 lbs
Glue	2 lbs
Potassium dichromate ...	$\frac{3}{8}$ oz.
Water	10 gallons

To make this mixture 30 pounds of lime are placed in a tub and 6 gallons of water poured over it and allowed to slake. When the lime begins to boil add 2 more gallons of water gradually so as to keep the slaking mixture always as hot as possible. Then 2 pounds of glue are put into a pail with one gallon of cold water and occasionally stirred. After the lime has slaked and become quite cold, which takes 6–12 hours, a gallon of hot water is added to the glue which then immediately goes into solution. This is then added to the lime, well stirred and filtered through a sieve with 16 meshes to the inch into the spraying machine.

Lastly the potassium dichromate previously dissolved in a small quantity of water is poured into the machine and stirred up. The mixture is then ready for application.

Where the lime is very good the amount might be reduced to 25 lbs as 30 lbs of good lime sometimes makes the mixture too thick for easy straining. It is important to soak glue first in cold water as a direct application of hot leads to the formation of intractable lumps. Where it is desired to avoid the use of hot water, as in continuous spraying, the following procedure may be adopted.

One lot of lime is slaked with water and allowed to cool as above described. Then a second lot is made up and by means of the heat evolved the glue for the first lot can be dissolved by standing the pail of soaked glue in the hot lime. It only needs a temperature of about 100° F. to dissolve glue that has been previously soaked and this is easily obtained by this method.

This lime-glue-dichromate mixture has been tried against ordinary lime-wash at Long Ashton and has given decidedly superior results. An application to an apple tree stopped aphis hatching to such an extent that hardly an aphis was to be seen on it throughout the season though control trees were very badly attacked. The tree stood out all the season from its fellows by the healthy green uncurled leaves and at the end of the season by its very numerous well-developed fruit buds.

Hide glue can be obtained in hundredweight quantities at 4½*d.* a pound so that the cost of the spray per 10 gallons works out as follows:

	s.	*d.*
Lime 30 lbs @ 1*s.* per cwt.		3
Glue 2 lbs @ 4½*d.*		9
Potassium dichromate ⅜ oz. @ 6*d.* per lb. ...		⅐
Total	1	0⅐

giving an approximate cost of 1¼*d.* per gallon.

TABLE I. *Indoor Trials.*

	Parts by weight including in each case water 100		Remarks	Resulting coat
(1)	Lime	20	Control wash	
(2)	Lime .... Resin	20 ⅛	Resin added during slaking Resin dissolved in caustic soda	About same as control Soft and brittle
(3)	Lime .... Casein	20 ⅛	Casein added during slaking	Softer than control
(4)	Lime .... Starch	20 ⅛	... ...	Hard but very brittle
(5)	Lime .... Dung	20 2	Dung macerated first with a little caustic soda	Soft and thin
(6)	Lime .... Filter-paper . Caustic soda.	20 *xs* *xs*	... ...	Well matted coat firm but uneven
(7)	Lime .... Filter-paper . Caustic soda. Starch	20 ½ ¼ ½	... ...	Not quite so firm as (6) but thick and good
(8)	Lime .... Newspaper ... Caustic soda. Starch	20 ½ ⅛ ½	... ...	About the same as (7)
(9)	Lime .... Washed pulp	20 1	... ...	Moderately hard, not flaky Firm
(10)	Lime .... Unwashed pulp	20 1	... ...	Softer than (9)
(11)	Lime .... Washed pulp Starch	20 1 1	... ...	Irregular in thickness, firm but fairly hard
(12)	Lime .... Unwashed pulp Starch	20 1 1	... ...	Not quite so firm as (11)
(13)	Lime .... Boiled oil .... Starch .... Caustic soda.	20 4 2 ·4	Starch treated with the caustic soda and the boiled oil stirred in and whole mixture added to the lime	Not enough boiled oil to affect the coat

TABLE II. *Outdoor Trials.*

Figures indicate parts by weight and include in each case water 100

	Lime	News-paper	Caustic soda	Starch	Washed News-paper pulp	Boiled oil	Remarks	Results
(1)	20	—	—	—	½	—	—	Fair coat
(2)	20	—	—	½	½	—	—	Rather better than (1)
(3)	20	—	—	1	½	—	—	Resisted rain fairly well
(4)	20	—	—	1½	1	—	Kept 12 hrs before application	Rather spotty but only slightly flaky
(5)	20	—	—	—	½	—	Applied fresh	Very flaky
(6)	20	—	—	—	½	—	Kept 6 hrs before application	Not nearly so flaky as (5)
(7)	10	½	⅛	3	—	—	—	Slightly flaky
(8)	10	—	—	3	½	—	—	Powdery
(9)	10	—	·4	2	—	2	—	Too thin

SOME OBSERVATIONS ON THE EGG OF *PSYLLA MALI.*

By A. H. LEES, M.A.,

Plant Pathologist, University of Bristol, Agricultural and Horticultural Research Station.

For some years it has been known that the egg of the Apple Sucker, *Psylla mali,* hatches at slightly different times on different varieties of apple trees. Theobald in his *Insect Pests of Fruit* makes the following remarks:

"Another point of great interest concerning the eggs is the irregularity with which they hatch out. The date not only varies in different localities and in different years but during the same season in the same plantation. They incubate at different periods according to the variety of apple upon which they are situated. This may be due to the heat generated by the flow of sap regulating their hatching to the time of the bursting of the buds."

So close was the correspondence between the date of bud bursting and egg hatching that it appeared as if there might be some intimate relation between the egg and the host plant. Investigations were therefore begun by the author in 1911 but owing to lack of opportunity could not be continued till 1915. A description of the egg may be found in Theobald's *Insect Pests of Fruit* where a certain process of the egg is mentioned and figured. He describes the egg thus:

"The ova when first laid are almost white, then they become creamy yellow and later assume a faintly rusty-red hue before hatching. Furley describes them as becoming a pale yellow-red also. They are elongated oval in form, somewhat pointed at the ends, one of which is produced into a thin process which is apparently curled under the egg and cannot easily be seen unless the ovum is very carefully removed. What this process is for we do not at present know."

Under ordinary circumstances this is the only process that can be seen when eggs are taken off a twig for observation. For further investigation it was found necessary to make use of special methods of preparation.

METHODS OF PREPARATION.

Treatment with 10 *per cent. caustic soda.* If the egg-covered twig be immersed for several days in cold 10 per cent. caustic soda it is possible to withdraw the egg from the plant tissues in which it is imbedded without injury to it. The soda softens the plant tissues and in the space of a few days does not destroy the structure of the larva. In some cases by careful manipulation with mounted needles it was possible to withdraw the larva with its accompanying membranes from the egg shell but usually the larva came away with only one membrane.

Treatment with bleaching powder solution. In order to study the relation of the egg membranes with more certainty it was found necessary to use some substance that would attach chitin. The most convenient was a mixture of concentrated bleaching powder solution and 10 per cent. caustic soda in about equal parts. This mixture when filtered from the precipitated hydroxide of calcium gives what is practically Eau de Labarraque with excess of soda. By judicious use it is possible to attack the outer chitinous membrane so as nearly to dissolve it without materially injuring the internal structures.

Mounting. In most cases concentrated carbolic acid with a small proportion of added glycerine was used. This mixture clears well and gives preparations sufficiently permanent for observation.

MATERIAL.

As it was desired to study the relation of parts of the larva and egg it was necessary to collect material just before hatching commenced. It was accordingly collected in the middle of April and preserved in alcohol.

One difficulty in this investigation was the very small number of perfectly formed advanced embryos that could be found. Other writers have pointed out the frequent presence of numerous empty egg shells, but in addition to this I found large numbers of eggs that appeared fairly normal to the naked eye but which proved on further examination to be much retarded in development. It is probable that these eggs were arrested in growth since the normal egg was just about to hatch, but at the same time it is of course possible that further development might have taken place though this seems unlikely in view of their very backward condition.

The eggs are laid in greatest number at the base of the fruit spurs, often sheltering under the edge of a scale scar; frequently one side of the spur has more eggs laid on it than the other. I have never observed that the eggs are orientated in a line parallel to the axis of the twig as mentioned by Awati (*Ann. App. Biol.* Vol. I, Nos. 3 and 4, p. 248).

As a rule there are but few eggs laid on the vegetative shoots of one year's growth. This occurs only in cases where there is considerable crowding of eggs on the fruit spurs.

DESCRIPTION OF THE EGG.

By using the caustic soda treatment combined with the use of bleaching powder solution it is possible to distinguish three membranes round the developing larva, which for convenience of description may be called the outer, middle, and inner membranes.

The outer membrane. This is comparatively thick and has a sculptured surface (Fig. 2). It has two processes. One is situated at the pointed end and is solid, being continuous with the thick chitin of the outer membrane. It is hyaline and lacks the irregularity of the sculptured surface which is present on the rest of the outer membrane. It is scarcely possible that it has any influence on the hatching of the egg and appears as if it were pulled out at the time of secretion when the chitin was still soft, in much the same way as a glass rod heated in a flame and pulled forcibly apart leaves a tail of glass (Fig. 2).

The other process is hollow and is inserted through the back into the cortex of the twig where it ends blindly (Figs. 1 and 3).

There is a certain amount of disturbance in the cortex as the cells immediately round the process are darker in colour as if cork had been formed as the result of irritation.

This process certainly serves to anchor the egg and it is possible that by its penetration into the tissues some kind of physiological reaction is caused between the egg and the tree. Of what nature this reaction is it would be impossible to say with any certainty.

It is at any rate certain that eggs situated on early leafing trees hatch correspondingly earlier than those on late leafing trees. Earlier hatching is therefore associated with earlier rise of sap in the tree. It would therefore appear not impossible that the sudden increase of sap in the vicinity of the process would cause an equally sudden increase of pressure inside the egg which might be just enough to cause hatching if the larva were in an advanced enough state.

The middle membrane. This is thin and is apparently not chitinous as picric acid does not stain it. It has no sculpturings like the outer membrane. It possesses a hollow thin-walled process which follows the position of the outer membrane process, reaching in the normal position about two-thirds way down it (Fig. 3). On treatment with reagents some contraction usually takes place so that it may be partially withdrawn (Fig. 1).

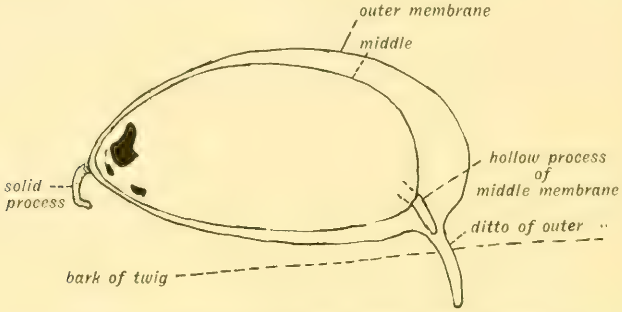

outer membrane
middle
hollow process of middle membrane
solid process
ditto of outer
bark of twig

Fig. 1. Outline of egg membranes and processes.

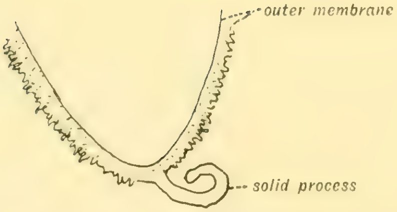

outer membrane
solid process

Fig. 2. Outer membrane.

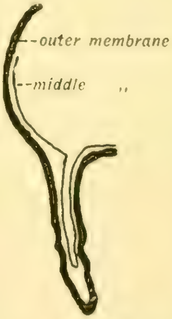

outer membrane
middle ,,

Fig. 3. Hollow process (highly magnified).

The inner membrane. This is extremely thin and is easily destroyed when preparing specimens, so that unless some care is exercised it cannot be seen. It has no process, but at the anterior end are two tubercles and a peculiar modified area (Figs. 1, 4, 5). The larger (Fig. 6 A) is dorsally situated and doubtless is the chief agent used for breaking the outer membrane. The smaller is ventrally situated and does not appear to be in such a position as to be of service in bursting the outer shell.

At the most anterior end of the inner membrane is a peculiarly modified area. Viewed under a high power it appears as a series of radiating thickened lines (Fig. 6 B). It has the appearance of being specially formed for ensuring the bursting of the membrane at the time of egg hatching.

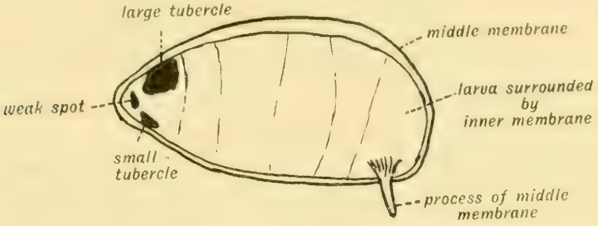

large tubercle
middle membrane
weak spot
larva surrounded by inner membrane
small tubercle
process of middle membrane

Fig. 4. Egg divested of outer membrane.

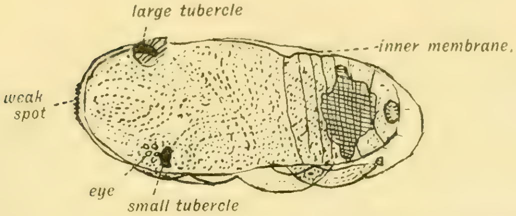

large tubercle
inner membrane.
weak spot
eye
small tubercle

Fig. 5. Egg divested of outer and middle membranes.

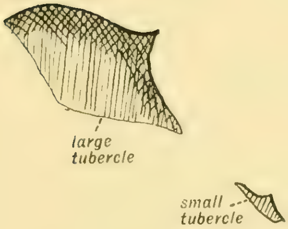

large tubercle
small tubercle

Fig. 6 A. The tubercles.

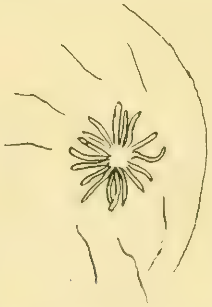


Fig. 6 B. Weak spot in inner membranes.

That these tubercles are not on the larva, as suggested by Awati, but on the inner membrane is shown by the fact that a newly hatched larva or one in which hatching has been induced artificially is perfectly free from them (Fig. 7).

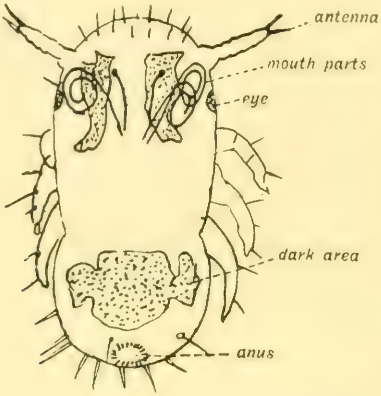


Fig. 7. Larva removed from egg membranes.

Position of larva in egg membranes. In Fig. 8, the original of which was prepared by carefully treating an egg with the bleaching powder mixture, the larva is shown *in situ.* The outer membrane is nearly dissolved away and the process of the middle membrane can be seen partially withdrawn from its normal position. The larva lies with its anterior end at the pointed end of the egg with its ventral surface towards the twig. Its posterior end is towards the hollow process and its closely packed legs are situated immediately over it.

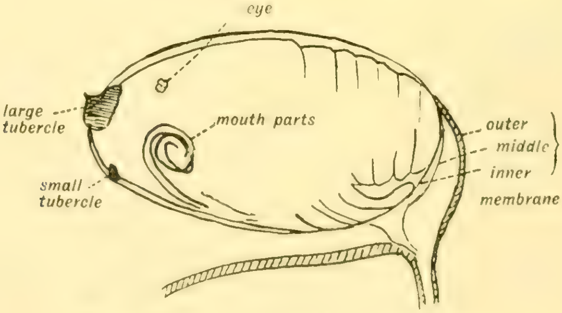


Fig. 8. Relation of larva to membranes.

At first it was thought that possibly the mouth parts went down the hollow process and served as a physiological connection between the apple tree and the egg. When examining specimens of the entire egg some straight lines could be seen in the hollow process of the outer membrane, but subsequent more careful examination showed these to be the two edges of the hollow process of the middle membrane.

The larva is entirely shut off from the hollow processes by the inner membrane and the mouth parts in every specimen examined were found curled up anteriorly and ventrally. On hatching the outer and middle membranes are left with their processes imbedded in the twig.

Morphology and function of egg membranes. In most insect eggs two membranes can be observed, the inner, or oolemma, and the outer, or chorion. The latter is often divided into exo- and endochorion connected by chitinous trabeculae, the ends of which in surface view give rise to the sculpturings of the outer egg shell. It is clear that in the case of *Psylla mali* the inner membrane is the oolemma and the outer is the chorion. It is difficult to see what the middle membrane corresponds to.

The relation of the egg coverings is peculiar and suggestive. If the object were simply to secure an anchoring mechanism the outer process would seem sufficient. It is possible, however, that there may be some physiological connection between the egg and the apple twig. The facts already quoted of the close relation between the time of hatching and the time of bud bursting in different varieties of apples would seem to suggest this. Until more direct evidence is obtained, however, it would be premature to press this hypothesis.

SCIARA TRITICI, COQ. A FLY INJURIOUS TO SEEDLINGS.

By F. W. EDWARDS, B.A., F.E.S.,
and C. B. WILLIAMS, M.A., F.E.S.

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Until comparatively recent years few or no instances were known of damage to plants by fly larvae of the genus *Sciara,* and even in some recorded cases it was supposed that the injury caused by the *Sciara* larvae was only of a secondary nature, and that they only attacked parts of plants where decay had already begun to set in owing to the attacks of some other pest. Recent investigations by several observers in the United States have, however, shown that in a number of instances the damage must be directly attributed to the larvae of some species of *Sciara.*

No definite records have been published on this subject in Britain, and it has therefore been thought worth while to issue the following notes on a species of *Sciara* which certainly causes damage to potted plants in this country. Added interest is given by the fact that the species in question has not previously been recorded in Great Britain, or even in Europe, though it is quite uncertain where it had its original home.

In the autumn of 1911 specimens of a *Sciara* were sent me for identification by Mr C. B. Williams, from Merton, Surrey, where they were damaging *Primula* seedlings. At that time I was unable to identify these specimens with any described European species, and they were in consequence put on one side. The question was reopened in June of the present year (1915) when living specimens of the same species were received from Mr W. H. St Quintin, of Rillington, Yorks, whose young orchid plants had suffered from their attacks. I then studied the literature more carefully and found that the specimens were without any doubt identical with the *Sciara tritici* described by Coquillet from North America in 1895.

Coquillet made positive statements as to the injury caused by the larvae to young wheat plants. He says (*Insect Life*, Vol. VII, 1895, p. 407): "On March 17, 1885 a large number of adults issued from a jar containing plants of this kind that had attained a height of from 6 to 8 inches. They already indicated an unhealthy growth by a more or less yellowish appearance, and an examination of their roots revealed the fact that these had been severely injured by the larvae, many of which were still present, and were observed to feed upon the roots and interior of the stems both below the surface of the soil as well as in the interior of the stems a short distance above the surface. As many as eight larvae were sometimes found in one of the stems, and they had also penetrated the kernels of wheat from which the plants sprang; many of the smaller rootlets had also been devoured, or more or less injured, by them."

Mr St Quintin was equally definite in attributing the damage to the *Sciara* larvae[1], as the following extract from his letter will show:

"It is causing great trouble and loss to raisers of exotic orchids and seems to be on the increase. The books written only a few years back do not allude to it amongst the insect pests which trouble orchids under cultivation. Certainly here, though we have been raising orchids (from seed) for about 8 years, until the last three or four we have never noticed it. It is particularly troublesome this spring, but it seems to fly all the year round, at any rate whenever we have young orchid plants at a particular stage, we find it in our seedling house. This house is kept very moist, and with a temperature of from 70°-80° F. according as the day is dull or sunny.

"When the little plants have acquired the real root, the fly does not trouble them, but at the very early stage when the plant draws its nourishment from root-hairs it cuts these off and starves the plant. When it has destroyed all the little plants in a pot it turns to the *Sphagnum* moss and devours that.

"Fumigation kills the insect when it is on the wing, but it appears to emerge at all hours of the day, and does not mind the fumigation in the larval and pupal stages.

"It seems a question whether the fly came with the *Osmunda* fibre, which has within the last few years been more or less used in the compost for raising orchids from seed, or in the *Sphagnum* moss, which has always been used since the first experiments in raising epiphytic orchids. The *Osmunda* fern fibre comes from N. America, the *Sphagnum* used is British."

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[1] Some of these larvae have been handed to Mons. D. Keilin, who will describe them in his forthcoming work on the early stages of the Diptera.

In the case of these orchids the possibility should not be overlooked that at least some of the damage may be caused by springtails (*Collembola*). These insects were present in large numbers in the contents of some pots sent to the British Museum by Mr St Quintin, and it is worth recalling that in 1902 Prof. F. V. Theobald recorded a case of injury to seedling orchids by springtails in Surrey (vide *First Report on Economic Zoology*, Brit. Mus. Nat. Hist., p. 110). In this last-mentioned case specimens of a Sciarid fly (*Zygoneura* sp.) were found in addition to the *Collembola*, but Theobald had little doubt that the damage was really caused by the latter. In Mr St Quintin's pots both the *Collembola* and the *Sciara* larvae were much more numerous in the *Osmunda* compost than in the *Sphagnum*.

Prof. Theobald has recently had a species of *Sciara* injuring tomato seedlings at Wye, and at my request he kindly sent me specimens of the fly concerned; contrary to expectation they proved not to be *S. tritici*, although they were not in sufficiently good preservation to be determined accurately.

Sciara tritici is a small fly about 2 mm. long, differing from most of its congeners in the reddish coloration of the thorax, somewhat darker towards the margins when viewed from above; the abdomen is rather darker than the thorax. Its most obvious distinguishing feature however—by which it may be separated from all the other members of the genus so far described—is the possession of a whitish-yellow stripe on the dark brown pleurae, connecting the bases of the front and middle coxae. There is also a whitish spot below the shoulder. It belongs to the group II.A.1.C.*b* of Winnertz's monograph. Coquillet, in the paper cited, gives a good description and figures the larva, pupa and adult; from these the species will be easily recognised, but it may be pointed out that the figure of the wing is not accurate, the subcostal cell being made much too narrow.

F. W. E.

THE BRITISH MUSEUM OF NATURAL HISTORY,
November, 1915.

Seedlings, particularly *Primula* and *Campanula*, under glass at this Institution, have, at various times during the past few years, suffered slightly from the attacks of small Dipterous larvae. The larva was of the Mycetophilid type, very long and slender with a shining black head, and adults bred out and captured in the hot-houses have been identified by Mr Edwards as *Sciara tritici* as mentioned above.

The larvae are found usually below the surface of the soil where they eat the roots and the collar of the seedlings. Frequently the root was eaten right across below the collar so that the dying rosette of the seedling would be found resting on the soil unattached to the roots. Sometimes a cotyledon or a small leaf which was touching the surface of the soil would be damaged on its lower surface. Pupae were found also just below the surface and the empty skins projecting slightly above having either worked their way there before emergence or been dragged to this position by the emerging fly. The adults were found commonly running about on the surface of the soil, on the sides of the boxes and pots containing the seedlings and occasionally resting underneath the boxes. They ran actively, were reluctant in taking to flight and their flight was not powerful.

Three larvae, quite large on the 9th January, 1912, were given a small piece of root of a larger *Campanula* which they fed on readily, removing the softer tissues and leaving the fibrous vascular bundles. They spun a slight web and pupated on the same web on the 19th, 20th, and 21st of January. The pupa was pale shining yellow and 2 mm. long by 0·7 mm. broad. A day or two after pupation the eyes began to darken. After four days the anterior end of the pupa darkened and this gradually spread till the whole, just before emergence, was dark brown. One adult emerged on the 25th and two on the 27th, giving a pupal stage of 6–7 days.

In emergence the pupal skin split along each side of the antennae, leaving the covering of these free except at the base where it was joined to the ventral surface of the pupa case.

Another dipteron, *Scatella quadrata*, is nearly always associated with *Sciara tritici* but as yet no damage has been traced to it.

A *Sciara* larva, indistinguishable from the above, was found *out of doors* in the root of an old *Campanula*. It pupated among the fibres of the root but unfortunately died in the pupal stage.

The pest has been kept under control by regular fumigation of the houses, chiefly with fumigants containing nicotine. It was also largely destroyed by the sterilization of the soil by heating which was tried experimentally for some time. This method, however, was abandoned as it was not found satisfactory for other reasons.

It is suggested by Mr St Quintin (see above) that the species in his case was introduced from the United States in *Osmunda* fibre. In our houses there were a few orchids growing in *Osmunda* fibre and it is possible that they might have come in this from some other locality in England. It is probable that it will be found widely distributed in greenhouses in this country.

C. B. W.

THE JOHN INNES HORTICULTURAL INSTITUTION,
MERTON, SURREY.
November, 1915.

ON A CASE OF RECOVERY FROM MOSAIC DISEASE OF TOMATO.

By W. B. BRIERLEY.

(*Laboratory of Plant Pathology, Royal Botanic Gardens, Kew.*)

During recent years the attention of plant pathologists has been increasingly directed to the study of certain diseases of obscure etiology, which have for convenience been classed apart under the term "physiological." Of these one of the most interesting yet least understood is Mosaic disease, often known as "Frenching," "Calico" or "Mottle Top," and found in a variety of plants chiefly members of the Solanaceae. Great attention has been devoted to mosaic disease of tobacco, but little focused on the study of this malady in tomato. In 1908, however, Clinton[1] published the important fact that the mosaic disease of tobacco is communicable to healthy tomato plants and *vice versa*; and this conclusion, although opposed by Westerdijk[2] in an important paper appearing two years later, is generally accepted.

Excellent recent accounts of the past history and present position of the question of mosaic disease will be found in the papers of Melchers[3], Allard[4] and Clinton[5], and further discussion here is unnecessary.

The interest of the present note lies in the exceedingly rare occurrence of recovery from the disease.

In 1898 Beijerinck[6] stated, but without entering into any details, that in some instances plants apparently showed a temporary recovery.

Woods[7], four years later, writes: "The mosaic nature of the trouble and the fact that under some conditions the plants may grow out of the disease."

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[1] *Rept. Conn. Agr. Expt. Sta.* (1908).

[2] *Mededeel. Phytopath. Lab.* "*Willie Commelin Scholten,*" Amsterdam, I. (1910).

[3] *Ohio Nat.* XIII. No. 8 (1913).

[4] *Bull. Torr. Bot. Club*, 41 (1914).

[5] *Rept. Conn. Agr. Expt. Sta.* (1914).

[6] *Verhandel. Kon. Akad. Wetensch.* Amsterdam, XI. 6/5 (1898).

[7] *U.S. Dept. Agr. Bur. Pl. Ind. Bull.* 18 (1902).

In 1910 Lodewijks[1] published the results of his investigations on mosaic disease. He covered the upper diseased portions of mosaic plants and at the same time exposed the lower apparently healthy leaves to light of different colours. Under these conditions he found that diffused light checked the disease, red light decreased it, and blue light completely cured plants of the malady. These very striking results still await confirmation.

Allard writing in 1914[2] states that "Some practical growers have claimed that the disease can be checked if taken at its first appearance by pulling affected plants until they are loosened from the soil." Of this he remarks "There is little in this view however to recommend its general adoption" and that "In the writer's experimental tests many thousands of affected plants have been kept under observation through all phases of the disease. In no instance has there been a case of actual recovery from true mosaic disease."

Allard's experience confirms that of practically all recent investigators of this malady.

Thus Clinton[3] writes: "A plant which once becomes infected remains so, and all subsequent new growth (at least that above the lowest infected leaf) usually, if not always, becomes calicoed." "The clause is placed in parentheses because we are not sure whether the calico virus is carried downward in the stem as far as, or as readily as it is carried upward. If not, it stands to reason that if plants were calicoed late in life by touching and infecting the upper leaves only, and some time later were cut off at the base with a sterile knife the resulting suckers would not so surely calico as those from plants whose basal leaves were calicoed. Some evidence along this line is shown by experiment No. 124, where the juice from an apparently healthy leaf at the base of a plant calicoed above, failed to infect another plant when applied to it."

Chapman[4], writing in 1913, states: "Once the disease appears on a plant, all subsequent growth will be mosaic to a greater or less extent."

Moreover any apparent cases of recovery in the past are rendered extremely improbable in view of the fact recently demonstrated by Allard[5] that the virus of the disease may be present in a highly infective condition, and yet produce no external symptoms. This fact, that an individual may be diseased and serve as a centre of infection, whilst itself not showing any signs of disease, is well known to animal pathologists but does not seem to have been previously applied to plants.

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[1] *Rec. Trav. Neerland.* VII. (1910).

[2] *U.S. Dept. Agr. Bull.* 40 (1914).

[3] *loc. cit.* (1914).

[4] *Ann. Rept. Mass. Agr. Expt. Sta.* Pt. XI. No. 31 (1913).

[5] *loc. cit.*

In the course of some preliminary investigations on the mosaic disease of tomato which are being carried out in the pathological laboratory at Kew, the freedom from disease of new shoots springing from a diseased stock was noted. The plant in question was one of two which were received on September 9th, from a grower in whose houses tomatoes have shown the disease for some years. The roots were shaken partly free from soil, and were found to be well-developed. The plants had been topped some time previously and were each bearing small clusters of immature fruit. The foliage was good but calicoed down to the lowest leaf. The plants were posted to the laboratory and on receipt some two days later were immediately potted in light loamy soil and placed on the floor in a cool glasshouse.

One plant shortly died and was destroyed. Of the other all the leaves and the upper portion of the shoot withered and were cut away. Three new shoots however developed, all originating on the stem well above the lower leaves and these appeared perfectly normal, *i.e.* were healthy shoots from a diseased stock.

Now two criticisms may be made which are vital to this observation. One concerns the condition of the original plant, for if the diagnosis of the malady as mosaic disease be a mistaken one, the observation has no interest. The second concerns the new shoots, for if the disease is not really absent from them but present in a latent form, the apparent recovery merely serves to confirm Allard's observation.

But it is the lack of a final criterion which is the fundamental difficulty in all investigations of mosaic disease, for where there is apparently no causal organism, the rules of Koch are inapplicable.

Concerning the first criticism—that the original plant possibly did not suffer from mosaic disease—two criteria only are available; one, the symptoms of the disease; and the other its infectious quality.

Now there is no tomato disease of known parasitic origin, which produces external symptoms conceivably to be mistaken for those of calico or mosaic disease. On the other hand chlorotic or etiolated plants, or those suffering from excess or deficiency of food and other materials in the soil, or from soil sickness, whilst often assuming a greyish or yellowish colour, never show the characteristic and peculiar blotching or mottling of calico.

The infectious nature of the malady from which the original plant suffered is a question only to be determined by experiment. The withered leaves which had been thrown away earlier were carefully collected, and those which had been attacked by various saprophytic fungi rejected. The few remaining portions were thoroughly and rapidly washed in sterile water, and then the moist fragments ground to a pulp in a watch glass and extracted with 0·25 c.c. of sterile water. This was filtered twice through two thicknesses of finest filter-paper to eliminate as many bacteria as possible and any fungal spores. The filtrate was then inoculated into the leaves, buds and stems of twelve seedling tomato plants, and as a control twelve other seedlings were similarly treated with sterile water and the pots placed on the same shelf in a glass house. Of the sixteen plants which survived Black Neck (*Phytophthora* sp. (*omnivora?*)), frost and the undue proximity of hot pipes, five out of nine inoculated with "virus" showed mosaic disease, whilst this was completely absent from the seven remaining controls. Considering the old withered condition of the original leaves, the treatment they received and the dilution of the "virus" derived from them, the fact that five out of nine plants should be calicoed, removes any doubt that the original plant suffered from this disease. As a further precaution new healthy seedlings were inoculated from the experimental plants and the mosaic condition obtained a second time, with again a total absence in the control plants.

The second criticism—that the "virus" is not absent, but merely latent in the new apparently healthy shoots—is again a question for experimental evidence only. The expressed sap of one of the shoots was inoculated into seedling plants, and controls were similarly inoculated with normal plant juice. No trace of mosaic disease was obtained in either series of plants; and a second set of seedlings inoculated from the first gave a similar negative result. There can therefore be no doubt that the new shoots arising from the calicoed stock were absolutely free from mosaic disease.

A further point of extraordinary interest arises out of the above experiments,—are the new shoots growing from the calicoed stock immune to the "virus" of the disease; *i.e.*, have they acquired immunity as the result of one attack?

To test this the two remaining shoots were inoculated with "virus," but unfortunately the plant was killed outright by frost ten days later, at this time showing no sign of disease. As the incubation period of the malady is variable, and rarely less than one week, this, perhaps the most interesting experiment, was a failure.

"WILT" OR "CROWN-ROT" DISEASE OF CARNATIONS CAUSED BY *FUSARIUM* SP.

By PAUL A. VAN DER BIJL, M.A., F.L.S.,
Mycologist, Department of Agriculture, Union of South Africa.

(With Plates XXXVII—XL.)

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Introduction.

Sturgis[1] in 1897 described a disease in carnations known commonly as "Die-back" or "Stem-rot," as due to a *Fusarium* sp. He first observed the disease on the *William Scott* variety growing in the Station greenhouse, and subsequent enquiry showed this trouble to be the cause of great loss of certain varieties.

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[1] Sturgis, Wm. C., "Preliminary Investigation on a Disease of Carnations," *Ann. Rep. Conn. Agric. Exp. Station*, Part III, 1897, pp. 175–181.

The disease he describes as showing first a yellowing of the lower leaves which later become dry and dead. As it progresses the whole plant becomes involved until finally death ensues. There was no sudden wilting of the plants; thus those affected resembled plants from which the water or food supply was gradually withdrawn.

In rare instances where only a portion of a plant appeared affected, he succeeded in checking the progress of the trouble by removal of the infected portion, but as a rule the appearance of the first symptoms indicated death to the plant sooner or later.

The stalk just below the soil showed discoloration and disintegration in the outer layers, and the cambium cells were partially destroyed, while permeating the wood and collecting in masses in the larger vessels and ducts was a fungous mycelium consisting of delicate colourless threads.

He finally obtained spores of the fungus on an old plant which had died from the trouble and thus determined the causal fungus as being a *Fusarium* sp.

Pure cultures were easily obtained by inoculating culture tubes with bits of the fungus-infected material.

Sturgis succeeded in inducing the disease in plants planted in pots, the contents of which after sterilisation were infected with the fungus. Other pots, the contents of which too were sterilised but into which the fungus was not introduced, served as controls. In a second series, the contents of the pots were not sterilised but otherwise treated as above. The disease also appeared in some of the control plants of the first series, and he attributed its outbreak to the presence of the fungus in the tissues of the original cuttings. In both cases where a decided outbreak occurred in the sterilised soil, the cuttings were obtained from a locality infested with the fungus and were rooted in compost from the same locality.

At the time of publication only one form of spore had been observed, a spindle-shaped or fusiform spore, pointed at both ends, hyaline, slightly curved, 3–5 septate and measuring $25\text{–}38\mu \times 3{\cdot}5\text{–}4\mu$. These spores were borne singly or in small clusters on the tips of the sides of short branches of the mycelium, and seen in mass presented a pale salmon pink colour.

Under unfavourable conditions these spores were observed to pass over into bodies resembling resting spores, which on germination produced a mycelium bearing the typical *Fusarium* spores.

Stewart[1] writing in 1898 states that under the popular names "Stem-rot" and "Die-back" have been confused two distinct diseases in carnations.

The one due to *Rhizoctonia* causes plants to wilt suddenly by rotting of the stem at or just below the surface of the soil. The cortex of diseased plants separates readily from the wood, the pith is attacked quite easily, becoming water-soaked in appearance and filled with fungous hyphae.

The other, caused by a *Fusarium* sp., attacks chiefly the stem and larger branches, discolouring the wood and killing the cortex but rarely causing a soft rot. The affected plants die gradually with yellowing or drying of the foliage. The fungus rarely fruits on the outside of the stem but does so frequently in the cambium and pith of stems long dead. Stewart[2] has also described a leaf-spot disease in carnations caused by a *Fusarium* sp. Of this disease he says: "A bunch of carnations of the variety *Emily Pierson* was quite seriously affected with a peculiar leaf spot, the spots varied in length from $\frac{1}{8}$–1 in. The smaller ones were elliptical but the larger ones occupied the entire width of the leaf and were irregular at the ends. They were covered with a pinkish-grey mold and irregularly dotted at the centre with the light yellow spore masses of a species of *Fusarium*. Many of the worst affected leaves were dying." The author found the *Fusarium* always originating in a rust pustule and, though not capable of infecting uninjured leaves, readily entering through the epidermis which had been broken by the rust fungus, and bringing about decay of the leaf tissue. He further remarks that inoculation experiments may show this fungus to be identical with the *Fusarium* causing stem-rot in carnations.

In conclusion, he states that the carnations were grown under conditions exceptionally favourable to the attack of fungi, but they did well and were free from disease with the exception of the *Fusarium* leaf spot and a moderate attack of rust.

The disease herein described has much in common with the "die-back" or "stem-rot" disease of Sturgis[3]. It, too, is caused by a *Fusarium* sp., but whether it is the same species in the two instances

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[1] Stewart, F. C., "Stem Rot Diseases of Carnations," *Exp. Station Records*, 1898–99, Vol. x.

[2] Stewart, F. C., "A *Fusarium* leaf-spot of Carnations." *N.Y.* (*Geneva*) *Agric. Exp. Station Bull.* 164, p. 219, Dec. 1899.

[3] Sturgis, Wm. C., *op. cit.* p. 1.

I should not at this stage like to predict, and the question can only be solved by a comparative study of the two fungi.

This disease exists throughout the Union of South Africa, but is particularly bad in the Province of Natal where carnations are being largely cultivated for market purposes.

The investigation was undertaken primarily to test the growth of the fungus in pure culture in the presence of various chemicals; thus it was hoped that some light would be thrown on practical methods of control. Field experiments thus far conducted are not very conclusive and further investigations will have to be carried out in the field.

It is my pleasant duty to express my indebtedness to Mr B. A. Bell, Krantzkloof, Natal. Not only did he place his plots at our disposal for experimental purposes, but at a time when I could with difficulty leave the laboratory he kept a full record of how the experiments were going and in time submitted a very complete report.

To Mr I. B. Pole Evans, under whose direction the work was undertaken, I am grateful for many helpful suggestions and advice.

The numbers and the word "Plate" bracketed after the colours refer to the colour chart *Répertoire de Couleurs* published by the Société Française des Chrysanthémistes.

A. THE DISEASE.

I. *Symptoms of Disease.*

The lower leaves of the plants die and the sides turn upwards, the colour changes to a sickly white and the leaves are more upright than are those of healthy plants. The upper leaves have a withered and shrivelled appearance, and soon the affected plant is dead. The bast of the stem in the affected area is most frequently soft and easily separated from the wood. Badly diseased plants, when pulled, usually break off in this region slightly below the soil. The xylem or wood just above this rotten area is brownish in colour and permeated by the threads of the causal fungus.

II. *Cause of the Disease.*

1. *Isolation of the causal fungus.* In January, 1914, a number of carnations in large tins suddenly started wilting with the symptoms given above. Small pieces of diseased tissue were sterilised in mercuric chloride (1 : 1000) and, after being washed in distilled water, were shaken up in sterilised melted beef-broth agar and poured into plates, which

were incubated at 25° C. After five days there was a pure white, fluffy, mycelial growth from the pieces of carnation, and on this growth spores typical of the genus *Fusarium*.

On two more occasions this *Fusarium* was isolated from wilting plants growing in the same tin. The fungus was also obtained from specimens submitted by Mr G. E. Ensor of Pretoria: the disease also causes serious monetary loss to carnation growers in the Province of Natal.

2. *Inoculation of carnations with the fungus in pure culture.* On the 17th February, 1914, carnation plants growing in tins at the laboratory were inoculated with the causal fungus thus: the soil was slightly removed round the stems of the plants, which were then punctured, just below the natural soil level, with a sterilised needle. Into the punctures were inserted pieces of the fungus growing in pure culture on beef-broth agar, and the soil was replaced. On the 27th February one of the inoculated plants had wilted with the usual symptoms. Pieces of diseased tissue of this plant were fixed in picroformol (formol 30 c.c., glacial acetic acid 5 c.c., water 20 c.c. and the mixture saturated with picric acid) and embedded in paraffin for further study. Beef-broth agar plates were prepared with pieces of diseased tissue and in these the same *Fusarium* developed. By the 4th March another of the inoculated plants had wilted with the characteristic symptoms mentioned above, and here too the same fungus was isolated.

In the same tin with the inoculated plants were left controls which were treated similarly to the inoculated plants in every way except that the fungus was not introduced into the punctures. These controls remained healthy.

Successful attempts at inoculation were also made by burying next to the plants cultures of the fungus. The carnations experimented on were again grown in large tins. The soil round the stems of the plants in one tin was removed; in the holes were placed cultures of the fungus on rice and the soil was then replaced. Four days later some of the plants showed a sickly appearance. On the seventh day one plant was noticed with lower leaves sickly white and upper leaves flaccid; and when this plant was pulled up it was observed to have the characteristic crown-rot. On the 13th day three more diseased plants were observed in this tin, and on the 17th the last plant had contracted the disease.

Plants growing in a tin next the inoculated tin remained healthy during this time.

During the seventeen days while this experiment was kept under observation there was a rainfall of 4·19 ins., and the night previous to placing the cultures in the soil ·47 in. of rain fell: 2·19 ins. fell within the first four days; 1·3 ins. between the 4th and 7th days. There can be little doubt that the rainfall was responsible in certain measure for the early appearance of the disease. Some of the diseased plants showed the presence of the fungus as a whitish growth on the part below the soil.

3. *The fungus in the tissues of the diseased plants.* A transverse section through the diseased area shows the fungus threads in the xylem or wood. The wilting is undoubtedly due to the fungus blocking up the vessels, decay is hastened by the entrance into the attacked region of various saprophytic bacteria, though cases were observed where bacteria were few and even altogether absent.

The fungus is also abundant in the cells of the pith and is able to invade the middle lamellae of the cells in the attacked region. The fungus also permeates the cells of the cambium, phloem and cortex, though in these regions it is less abundant than in the xylem and pith.

4. *Humidity as a factor influencing infection.* In the field it has usually been observed that the disease appears at its worst just after the first rains. This was found to be the case also with plants inoculated at the laboratory, and throws some light on the fact that plants inoculated during the dry season in some cases took a month or more before showing symptoms of the disease.

B. GROWTH OF THE FUNGUS IN THE LABORATORY.

I. *Growth on Various Media at* 25° C.

Banana plugs. (Pl. XXXVII, figs. 1, 2.) Inoculated 6. vii. 14.

9. vii. 14. Dense greyish, fluffy growth.

13. vii. 14. Growth firm and compact, in places as much as 3·5 mm. thick. Surface more fluffy than on potato. Chlamydospores, both intercalary and apical, vary greatly in size, 13·2–26·4μ diameter.

20. vii. 14. Growth where it lies on cotton wool produces a region about 2·3 mm. wide of a leaden grey (Plate 353. 3) and above this a narrow, very bright yellow region (Plate 35. 4). In places on the glass a honey yellow (Plate 35. 4) is evident. No conidia.

24. vii. 14. The light yellow region above has changed to a tan colour (Plate 317. 1). Numerous very small conidia.

Length	Breadth	Number of cells
16·5 μ	3·3 μ	4
14·85	4·75	4
19·8	4·75	4
24·75	4·75	—
23·1	6·6	—
14·85	3·3	3
19·8	3·3	4
13·2	4·75	2

Sweet Potato. (Pl. XXXVII, fig. 3.) Inoculated 15. vii. 14.

18. vii. 14. Dense grey, fluffy growth forming a felt on surface of medium.

20. vii. 14. On surface of water the fungus has formed a thick growth which where it comes into contact with the glass forms a leaden grey (Plate 353. 1) ring, above which is a very light yellow region. Chlamydospores, but as yet very few conidia.

23. vii. 14. Majority of chlamydospores have a diameter of 23·1μ; leaden grey ring darker (Plate 353. 3), yellow above changed to a chocolate brown (Plate 342. 1). Growth 3 mm. thick; conidia abundant.

Length	Breadth	Number of cells
33·0 μ	6·6 μ	6
26·4	6·6	4
26·4	4·95	4
36·3	6·6	5
26·4	6·6	4
26·4	6·6	5
19·8	6·6	2
19·8	4·95	3
29·7	6·6	6

Sterilised Carnation stems in Petri dishes. (Pl. XXXVII, fig. 4.) Inoculated 13. vii. 14.

17. vii. 14. Dense, slightly pinkish growth, on the stalk is decidedly fluffy, but surface more mildewy. Numerous conidia of various lengths and different septations.

Length	Breadth	Number of cells
36·3 μ	6·6 μ	6
37·45	6·6	6
33	6·6	5
34·65	6·6	5
33	6·6	5

24. vii. 14. Colour on undersurface, especially in dried up plates, honey yellow (Plate 35. 4). Growth greyish rather than pink.

Sterilised Carnation leaves. (Pl. XXXVIII, figs. 5 and 7.) Inoculated 13. vii. 14.

18. vii. 14. Greyish mildewy growth (no pink). Chlamydospores intercalary, apical, or on short branches, majority 13·2–19·8μ diameter. Conidia of various lengths and septations.

Length	Breadth	Number of cells
26·4 μ	4·95 μ	5
26·4	6·6	4
26·4	6·6	5
19·8	4·95	3
28·05	4·95	4
24·75	4·95	3
26·4	6·6	3

24. vii. 14. On undersurface of dried up plates colour in places honey yellow (Plate 35. 4).

Potato plugs. (Pl. XXXVIII, fig. 6.) Inoculated 6. vii. 14.

9. vii. 14. Dense cottony pure white growth forming a felt-like layer 1–2 mm. thick. Mycelium swollen and macroconidia. Chlamydospores vary greatly, usually 23·1μ diameter. Conidia measure from 26·4–49·5μ in length, fusiform, septa 0–5.

18. vii. 14. On side of tube where growth comes into contact with glass, colour in places honey yellow (Plate 35. 4); on upper region of slant colour yellowish grey, below this growth more fluffy, pure white and pierced by small raised hummock-like bodies at the apex of each of which is a water-drop.

25. vii. 14. In places where growth rests on cotton-wool a leaden grey (Plate 353. 4) ring; in same neighbourhood colour in places dark chocolate brown (Plate 342. 1). Raised region light pink tinge and as much as 8 mm. thick.

Beerwort agar plates. (Pl. XXXVIII, fig. 8.) Inoculated 9. vii. 14.

18. vii. 14. Dense greyish mildewy growth, which on surface of medium is felt-like. Growth spreads all through dish. Reverse Petri dish colour yellow. This colour starts in the centre of a colony and spreads outwards.

21. vii. 14. Colour bottom of Petri dish, peach-red (Plate 98. 4). Majority of chlamydospores 16·5–26·4μ diameter.

25. vii. 14. Growth grey (Plate 359. 1) with, in places, a pink tinge, raised 2 mm. Reverse Petri dish madder-brown (Plate 334. 4). Conidia few, small, and general shape figured on banana.

Length	Breadth	Number of cells
14·35 μ	4·95 μ	2
26·4	6·6	4
26·4	4·95	4
19·8	6·6	3
19·8	6·6	4

Potato agar. (Pl. XXXIX, fig. 9.) Grey growth which in the centre is thick and fluffy-looking; about 3 mm. thick; 5·6 mm. from edge the growth is only raised about 1 mm. and radiates outwards. Colour on reverse of Petri dish, buff (Plate 309. 1). Chlamydospores 13·2–23·1μ diameter.

Measurements of conidia:

Length	Breadth	Number of cells
34·65 μ	6·6 μ	4
29·7	6·6	1
23·1	4·95	2
23·1	6·6	2
33·0	6·6	1

Liquid beerwort tubes. (Pl. XXXIX, fig. 10.) Inoculated 13. vii. 14.

20. vii. 14. Pure white cottony growth on surface of liquid; this growth creeps up the side of the tube. In one tube the following regions were evident:

(1) A leaden-grey ring (Plate 353. 1) about 3 mm. wide.

(2) Above this a rosy-flesh (Plate 134. 1) about 2 mm. wide.

(3) A very light yellow about 1 mm. wide.

(4) Above this pure white mycelial growth which creeps up the sides of the tube.

27. vii. 14. The regions in the tube above referred to are: (1) leaden-grey (Plate 353. 4); (2) rosy-flesh (Plate 134. 4); (3) mineral-brown (Plate 339. 4); (4) pure-white with yellowish places just above (3). Chlamydospores 14·85–26·4μ diameter.

Uschinsky's fluid in tubes. (Pl. XXXIX, figs. 11 and 12.) Inoculated 13. vii. 14.

20. vii. 14. In two tubes delicate mycelial growth in liquid. In remaining tube this growth has come to the surface where it forms a thick, pure white cottony growth, which climbs up the sides of the tube and rests on a light grey band.

27. vii. 14. The tube above shows the following regions in surface growth:

(1) Leaden-grey ring (Plate 353. 4).
(2) Above this mineral-brown (Plate 339. 3).
(3) Mycelium creeping up sides pure white excepting just above (2) where it is light yellow. Hanging from (1) into the liquid are two hard, wart-like bodies (above is a small pocket arrangement). A section through one of them showed nothing of peculiar interest. Chlamydospores abundant in this region. Mycelium, etc., coloured and oil globules frequently large.

Solution N + agar plates. (Pl. XL, fig. 13.) Inoculated 18. vii. 14.

22. vii. 14. In first two plates thick greyish felt-like growth covering whole Petri dish, furrows where originally separate colonies come into contact. Later plates colonies greyish, thick, dense in centre, towards periphery mildewy with a light pink tinge, raised 1–15 mm.

29. vii. 14. Growth creeps up sides of Petri dishes, mildewy. Colour on reverse of Petri dishes: (1) where growth touches glass, carrot-red (Plate 55. 1); (2) where growth is raised, mineral-brown (Plate 339. 4). Chlamydospores 19·8–33·0μ diameter.

Conidia:

Length	Breadth	Number of cells
26·4 μ	6·6 μ	4
29·7	4·95	4
26·4	6·6	4
23·1	6·6	4
26·4	4·95	—
29·7	6·6	6
26·4	6·6	6

Raulin's fluid. (Pl. XL, fig. 14.) Inoculated 13. vii. 14.

20. vii. 14. In all tubes colourless mycelial growth in liquid. This growth has not yet reached the surface on 22. vii. 14. Tubes shaken up so as to get growth to the surface.

27. vii. 14. Surface growth good.

31. vii. 14. The following regions in all the tubes:

(1) Leaden-grey (Plate 353. 1) 4–7 mm. wide.
(2) Pure white growth creeping up sides of tube.
(3) Between the above—a very light yellow tint.

6. viii. 14. Majority of chlamydospores 16·5–19·8μ diameter. Following regions:

(1) Leaden-grey (Plate 353. 4).
(2) Rosy flesh (Plate 134. 4) 3 mm. wide.
(3) Pale buff (Plate 64. 4).
(4) Above this pure white creeping up sides of tubes. Growth below warty and of a fleshy colour.

Turnip tubes. (Pl. XL, fig. 15.) Inoculated 1. viii. 1914.

5. viii. 14. Growth dense, greyish, fluffy to cottony. Conidia sparse with septa indistinct.

Length	Breadth
16·5 μ	5·775 μ
18·8	5·775
19·8	4·95
21·45	4·95
19·8	4·95
18·15	6·6
16·5	4·95

Chlamydospores 13·2–19·8μ diameter.

21. viii. 14. Growth 3–4 mm. thick, in places on surface greyish yellow. In some tubes raised hummock-like white bodies. In places along the glass, bronze-yellow (Plate 34. 3). Just above cotton-wool there is most frequently a leaden grey ring (Plate 353. 4) which may be yellowish in patches. Conidia abundant.

II. *Growth in the Absence of Free Oxygen.*

To test whether the fungus is able to grow in the absence of free oxygen, fermentation tubes containing the following media were inoculated: Uschinsky's fluid, Raulin's fluid, liquid beef-broth, liquid beerwort. Two tubes of each medium were inoculated, by removing a small piece of the fungus on agar and pressing it past the bend of the fermentation tubes; another piece of growth was placed on the surface of the liquid in the open end. The pieces pressed past the bend in some ascended halfway only or after going right up came down again and stopped halfway.

Inoculated 13. vi. 14. *Incubated* 25° C.

		Result	
Medium	Dates of examination	Growth on surface	Growth from piece past bend
Uschinsky's solution	1. vii. 14	Vigorous	0
Raulin's fluid	1. vii. 14	Vigorous	0
Liquid beef-broth	1. vii. 14	Vigorous	0
Liquid beerwort	1. vii. 14	Vigorous	0

The results enumerated above clearly show that the fungus is unable to take its oxygen from the compounds contained in any of the four liquids used. As far as could be determined, the fungus appears to be strictly aerobic.

III. *Temperature Relations.*

Tubes of various media were inoculated with the fungus obtained from a beef-broth agar culture, and incubated at the temperature stated in the table below. Two tubes of each medium were incubated at each temperature.

Date of inoculation: 24. viii. 14.

		Media used and growth at different temperatures			
Temperature	Dates of examination	Beerwort slants	Rice tubes	Uschinsky's solution	Sweet potato tubes
0° C.	26. viii. 14	No growth in any of the cultures			
	28. viii. 14	No growth in any of the cultures			
15° C.	26. viii. 14	Good	No visible growth	Feeble	Good
	28. viii. 14	Vigorous	Feeble	Good	Vigorous
25° C.	26. viii. 14	Vigorous	Good	Vigorous	Vigorous
	28. viii. 14	,,	Vigorous	,,	,,
30° C.	26 viii. 14	Vigorous	Vigorous	Vigorous	Vigorous
	28. viii. 14	,,	,,	,,	,,
37° C.	26. viii. 14	Starting	Starting	Exceedingly feeble	Exceedingly feeble
	28. viii. 14	Feeble	Feeble	Feeble	Feeble
40° C.	26. viii. 14	No growth in any of the cultures			
	28. viii. 14	No growth in any of the cultures			

Note 1. 26. viii. 14. At 30° C. the growth was in every instance more vigorous than at 25° C.

Note 2. 28. viii. 14. Growth at 25° C. and 30° C. equally vigorous At 15° C. less than at 25° C., and at 37° C. less than at 15° C.

Note 3. 31. viii. 14. Very little difference between growths at 25° C. and 30° C. on the one hand and 15° C. on the other. At 37° C. no further growth and feeble as compared with growth at 25° C. and 30° C.

On 31. viii. 14 all the cultures were incubated at 30° C. with the results expressed in the table below.

		Media and growth of cultures exposed above at 0° C., 37° C. and 40° C., and from 31. viii. 14 at 30° C.			
Former temperature	Dates of examination	Beerwort agar slant	Rice tubes	Uschinsky's solution	Sweet potato tubes
0° C.	2. ix. 14	Vigorous	Vigorous	Vigorous	Vigorous
37° C.	2. ix. 14	In all cultures vigorous; about equal to above			
40° C.	2. ix. 14	No growth in any of the cultures			
	4. ix. 14	No growth in any of the cultures			

IV. *Growth in beef-broth agar of various alkalinities on Fuller's scale*[1].

Inoculated 18. v. 14. *Incubated* 25° C.

Reaction of medium on Fuller's scale	Dates of examination	Sodium hydrate (caustic soda)	Sodium carbonate (washing soda)
–15	20. v. 14	Vigorous	Vigorous
–25	20. v. 14	Vigorous	Vigorous
–35	20. v. 14	Good	Good
–40	20. v. 14	Good	Feeble
–45	20. v. 14	Feeble	Feeble

Inoculated 4. vii. 14. *Incubated* 25° C.

Note. As the agar did not set firm on the slant, the tubes were not stood upright.

Reaction of medium on Fuller's scale	Dates of examination	Sodium hydrate (caustic soda)	Sodium carbonate (washing soda)
–70	6. vii. 14	Good	Good
	8. vii. 14	Vigorous	Vigorous
–80	6. vii. 14	Feeble	Fair
	8. vii. 14	Good	Vigorous
–85	6. vii. 14	Feeble	Fair
	8. vii. 14	Good	Good
–95	6. vii. 14	Very feeble	Feeble
	8. vii. 14	Feeble	Good
–100	6. vii. 14	—	Feeble
	8. vii. 14	Very slight	Good
Control	6. vii. 14	Vigorous	—

In reacts – 70 F. to – 100 F. the growth in the presence of sodium hydrate was in every case less vigorous than in tubes containing sodium carbonate in which latter again the growth was less vigorous than in the controls.

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[1] Fuller's scale expresses the amount of normal acid or alkali required to neutralise 1000 c.c. of an alkali or acid solution. Thus –15 F. means that the medium is alkaline and that 1000 c.c. requires 15 c.c. of a normal acid to neutralise it. Similarly +15 F. implies that the medium is acid and that a 1000 c.c. of it will be neutralised by 15 c.c. normal alkali.

V. *Growth in Rice of various acidities*[1] *with Hydrochloric, Nitric and Sulphuric Acids.*

In acidities – 30 F., – 60 F. the growth was in three days as vigorous as in the controls. In hydrochloric and sulphuric acid – 80 F., – 105 F. the growth within three days was less vigorous than in the controls, but within five days they came up to the controls. Growth in acidities higher than – 105 F. is given in the tables below.

Inoculated 14. vii. 14. *Incubated* 25° C.

Reaction on Fuller's scale	Dates of examination	Nitric	Hydrochloric	Sulphuric
+ 110	17. vii. 14	Fair	Fair	Slight
	20. vii. 14	Vigorous	Vigorous	Vigorous
	28. vii. 14	Mouse colour[2] (360. 2) and canary yellow (17. 4)	Terra cotta (331. 4)	Slate grey (362. 2)
+ 120	17. vii. 14	Slight	—	Slight
	20. vii. 14	Vigorous	Vigorous	Vigorous
	28. vii. 14	Ashy grey (358. 3) Canary yellow (17. 4) Brown lake (336. 1)	Smoke grey (363. 1)	Smoke grey (363. 1)
+ 130	17. vii. 14	Slight	—	Slight
	23. vii. 14	Vigorous	—	Vigorous
	28. vii. 14	Canary yellow (17. 4) Mouse colour (360. 2)	Vigorous Terra cotta (331. 1)	Terra cotta (331. 1)
+ 140	17. vii. 14	Slight	—	Slight
	23. vii. 14	Vigorous	—	Good
	28. vii. 14	Canary yellow (17. 4) Ashy grey (358. 3)	Feeble	Vigorous
+ 160	17. vii. 14	Slight	—	—
	23. vii. 14	Vigorous	—	Feeble
	28. vii. 14	Canary yellow (17. 4) Dark chocolate brown (342. 4)	Feeble	Feeble

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[1] The acidity of the rice was determined by placing a known weight of ground rice overnight in a known volume of neutral alcohol. Next morning the alcohol was filtered off, titrated and the acidity determined on Fuller's scale. Five grams of the rice were then placed in a wide test-tube and 5 c.c. of water containing enough acid to produce the required reaction added. The tubes were sterilised on the slant in the autoclave at 110–120° C. for 15 mins. and then inoculated with the fungus mycelium by removing small pieces of agar with the fungus on it and mixing this with the rice.

[2] The colour is that noticed in contact with the glass and the numbers refer to the *Répertoire de Couleurs* published by the Société Française des Chrysanthémistes.

Inoculated 31. ix. 14. *Incubated* 25° C.

Reaction on Fuller's scale	Dates of examination	Nitric	Hydrochloric	Sulphuric
+170	2. x. 14	Starting	—	Starting
	7. x. 14	Vigorous Lake brown	—	Vigorous
	10. x. 14	Canary yellow (17. 4)	— —	Rosy flesh (134. 4)
+190	2. x. 14	Starting	—	Starting
	7. x. 14	Vigorous. Colour as above	Good	—
	10. x. 14	—	Rosy flesh (134. 4)	Good
+210	2. x. 14	—	—	Starting
	7. x. 14	—	—	Feeble growth from piece of agar on rice
	10. x. 14	—	—	No better
+220	2. x. 14	—	—	Feeble growth, starting
	10. x. 14	Feeble	—	—
+240	2. x. 14	No growth in any of the tubes		
	10. x. 14	No growth in any of the tubes		
Control	2. x. 14	Vigorous white growth. Colour on glass pearl grey (354. 4) with, in patches, canary yellow (17. 4).		
	10. x. 14	Colour on glass terra cotta (331. 1) and pearl grey.		

Note. On the rice kernels small black pycnidia-like bodies were frequently observed. No spores were found in them and they would appear to be of the nature of sclerotia.

VI. *Experiments in the Laboratory with various Soil Fungicides.*

The quantities stated in the tables below were added to 50 c.c. neutral beef-broth agar. The formalin, carbolic acid and hycol solutions were added after sterilisation and the flasks immediately inoculated. The other substances were added before sterilisation and the flasks then autoclaved. The flasks were inoculated by dropping into them pieces of the fungus on beef-broth agar and then shaking up well. All the cultures were incubated at 25° C. and controls in every instance incubated at the same time.

SERIES I. 1 c.c. *Formalin diluted with* 100 c.c. *Distilled Water.*

Dates of inoculation	Dates of examination	Quantities of 1 % formalin solution added to 50 c.c. neutral beef-broth agar							
		·3 c.c.	·6 c.c.	·7 c.c.	·9 c.c.	1 c.c.	1·5 c.c.	2 c.c.	2·5 c.c.
11. v. 14	13. v. 14	Good	Good	Good	Starting	Good	No growth		
	15. v. 14	As vigorous as controls					Slight growth just starting		
14. v. 14	18. v. 14	—	—	—	—	—	Good	No growth	No growth

SERIES II. 1 gm. *Carbolic Acid dissolved in* 100 c.c *Distilled Water.*

Dates of inoculation	Dates of examination	Quantities of 1 % carbolic acid added to 50 c.c. neutral beef-broth agar						
		·3 c.c.	·6 c.c.	·7 c.c.	·9 c.c.	1 c.c.	1·5 c.c.	2 c.c.
11. v. 14	13. v. 14	Good	Good	Vigorous	Good	Good		
	15. v. 14	Vigorous	Vigorous	Vigorous	Vigorous	Vigorous		
14. v. 14	18. v. 14	—	—	—	—	Vigorous	Slight	Less than 1·5
		2 c.c.	3 c.c.	3·5 c.c.	4 c.c.	5 c.c.		
26. v. 14	31. v. 14	Fair	Fair	Feeble	Slight growth from piece in agar	Very slight growth from piece in agar		
	6. vi. 14	—	—	Vigorous	—	Growth from piece in agar not yet reached surface		
						5·5 c.c.	6 c.c.	6·5 c.c.
9. vi. 14	15. vi. 14	—	—	—	—	No growth	No growth	No growth
	20. vi. 14	—	—	—	—	No growth	No growth	No growth

SERIES III. 1 c.c. *Hycol in* 100 c.c. *Distilled Water.*

Dates of inoculation	Dates of examination	Quantities of 1 % hycol added to 50 c.c. neutral beef-broth agar						
		·3 c.c.	·6 c.c.	·7 c.c.	·9 c.c.	1 c.c.	1·5 c.c.	2 c.c.
14. v. 14	18. v. 14	Good	Good	Good	Good	Good	Good	Good
		2 c.c.	3 c.c.	3·5 c.c.	4 c.c.	5 c.c.		
26. v. 14	31. v. 14	Vigorous	Vigorous	Vigorous	Slight	Slight		
	6. vi. 14	,,	,,	,,	Vigorous	Vigorous growth from piece in agar spreading over surface		
		5·5 c.c.	6 c.c	6·5 c.c.				
9. vi. 14	12. vi. 14	Fair growth from pieces in agar	Slight growth from pieces in agar	Slight growth from pieces in agar				
	15. vi. 14	Vigorous	Vigorous all over	Vigorous all over				

SERIES IV. *Crushed Quicklime.*

Dates of inoculation	Dates of examination	Weights of quicklime added to 50 c.c. neutral beef-broth agar						
		·1 gm.	·3 gm.	·5 gm.	·6 gm.	·7 gm.	·9 gm.	1 gm.
6. v. 14	9. v. 14	Good	No growth	Starting	Starting	No growth	No growth	No growth
	11. v. 14	Vigorous	Vigorous	Good	Good	Slight	Slight	No growth
	13. v. 14	—	—	—	—	—	—	Starting
		1 gm.	1·5 gms.	2 gms.	2·5 gms.			
2. vi. 14	5. vi. 14	No growth	No growth	No growth	No growth			
	8. vi. 14	,, ,,	,, ,,	,, ,,	,, ,,			
	12. vi. 14	,, ,,	,, ,,	,, ,,	,, ,,			

SERIES V. *Crushed Kainit.*

Dates of inoculation	Dates of examination	Weights of kainit added to 50 c.c. neutral beef-broth agar						
		·1 gm.	·3 gm.	·5 gm.	·6 gm.	·7 gm.	·9 gm.	1 gm.
6. v. 14	9. v. 14	Good	Good	Good	Good	Good	Good	Feeble
	11. v. 14	Vigorous	Vigorous	Vigorous	Vigorous	Vigorous	Vigorous	Vigorous
			1·5 gms.	2 gms.	3 gms.	4 gms.		
2. vi. 14	5. vi. 14	—	Vigorous	No growth	No growth	No growth		
	8. vi. 14 12. vi. 14	—	"	" "	" "	" "		

SERIES VI. *Lithium Nitrate.*

Dates of inoculation	Dates of examination	Weights of lithium nitrate added to 50 c.c. neutral beef-broth agar						
		·1 gm.	·3 gm.	·5 gm.	·6 gm.	·7 gm.	·9 gm.	1 gm.
6. v. 14	9. v. 14	Vigorous	Vigorous	Vigorous	Vigorous	Feeble	No growth	Feeble
	11. v. 14	—	—	—	—	Vigorous	Feeble	Feeble
		1·5 gms.	2 gms.	2·5 gms.				
2. vi. 14	5. vi. 14	Slight	No growth	No growth				
	8. vi. 14 12. vi. 14	Feeble	" "	" "				

SERIES VII. *Copper Carbonate.*

Date of inoculation	Dates of examination	Weights of copper carbonate added to 50 c.c. neutral beef-broth agar					
		·1 gm.	·3 gm.	·5 gm.	·7 gm.	·9 gm.	1 gm.
2. vi. 14	5. vi. 14	Vigorous	No growth	No growth	No growth	No growth	No growth
	8. vi. 14 12. vi. 14	"	" "	" "	" "	" "	" "

SERIES VIII. *Sodium Chloride (common salt).*

Date of inoculation	Dates of examination	Weights of salt added to 50 c.c. neutral beef-broth agar					
		3·5 gms.	4 gms.	4·5 gms.	5 gms.	5·5 gms.	6 gms.
3. vii. 14	5. vii. 14	No growth	No growth	No growth	No growth	No growth	No growth
	8. vii. 14	Vigorous	Vigorous	Good	Good	" "	" "
	10. vii. 14	—	—	Vigorous	Vigorous	Vigorous	

C. FIELD EXPERIMENTS.

Mr B. A. Bell of Krantzkloof, Natal Province, very kindly assisted in allowing field experiments to be carried out at his place. This place particularly suited our purpose as the soil was very badly infected since diseased plants had inadvertently been dug in.

The experimental plots were situated on a gentle slope, facing north. Each plot was about 70 × 40 ft. The soil was a sandy loam.

The area placed at our disposal was divided into six plots and these plots treated as follows:

Plot A. Crushed quicklime at the rate of two tons to the acre. The quicklime was applied as a surface dressing on the 21st September; raked in on the 1st October and again raked over on the 7th October.

Plot B. Control.

Plot C. Crushed quicklime at the rate of 3 tons to the acre. Applied and treated as for plot *A*.

Plot D. Crushed quicklime at the rate of 5 tons to the acre. Applied and treated as for plot *A*.

Plot E. Control.

Plot F. One per cent. formalin applied at the rate of 2000 gallons to the acre. The soil was watered with the formalin solution on the 29th September and raked over on the 1st and 7th October.

The planting of carnations was commenced on the 15th October and continued on the 16th, 17th, 21st, 22nd and 23rd. The weather remained good; cloudy and showery but not enough to stop work; the ground being kept just moist enough.

In planting each plot was divided into six sections and planted as under:

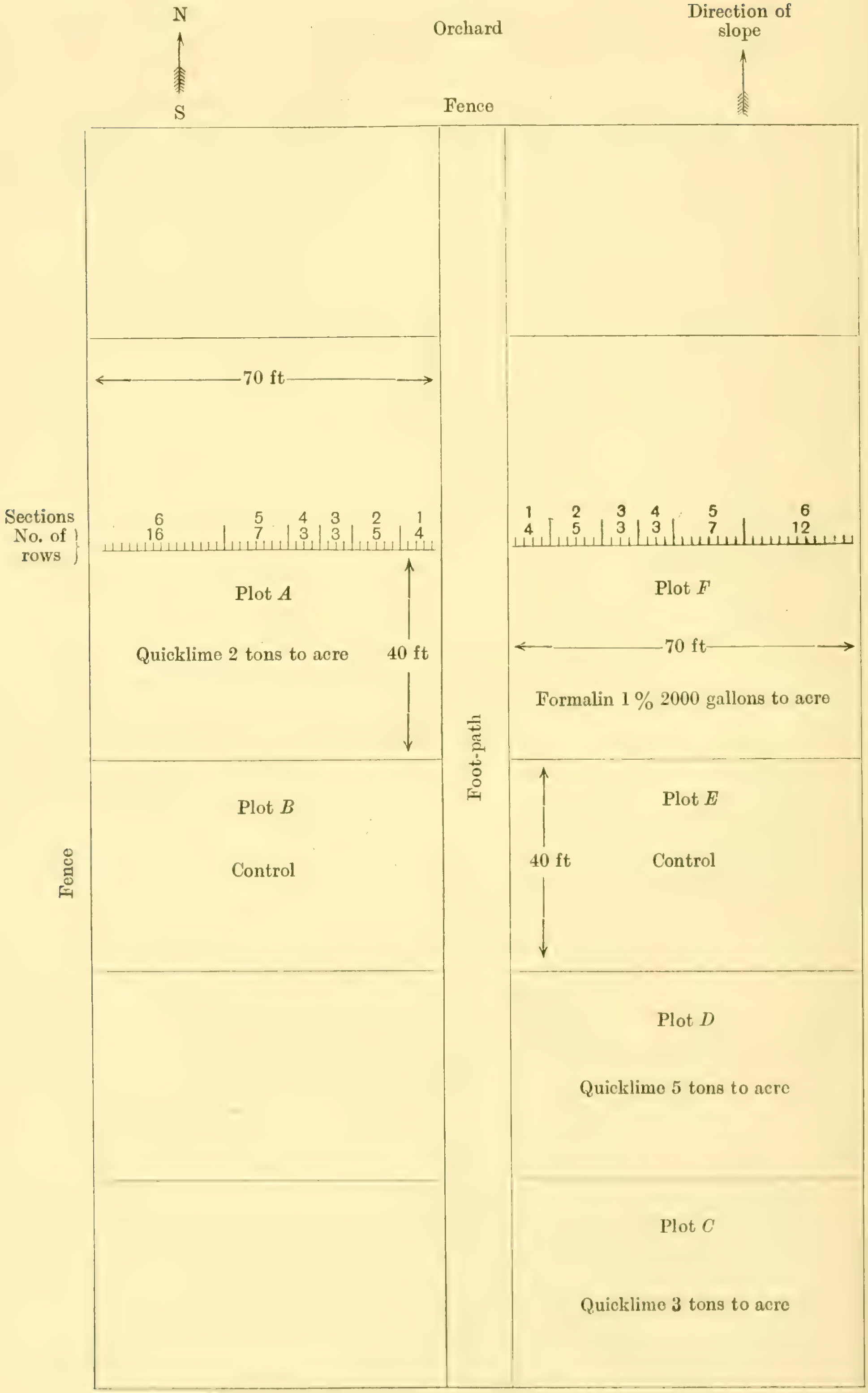
Section	Distinguishing feature	No. of rows
1	Pink tree, raised by Mr Bell	4
2	Pink tree[1], obtained by Mr Bell from a friend	5
3	Named varieties[2], own raising	3
4	Common red[3] (little value)	3
5	Pink tree, obtained from another friend ...	7
6	Pink tree mixed with common red and named varieties	
	Plots *A* and *B*	16
	Plots *C* and *D*	12
	Plots *E* and *F*	11

[1] An unhappy lot of plants from the beginning.

[2] The varieties were: Georgia, My Maryland, Andrew Carnegie, Mrs Henry Lawson, Regina, Lady Bountiful, La Mode, Verita.

[3] Very promising plants.

Below is a rough plan of the area experimented on.


N
S
Orchard
Fence
Direction of slope
Sections
No. of rows
6 5 4 3 2 1
16 7 3 3 5 4
1 2 3 4 5 6
4 5 3 3 7 12
70 ft
Plot A
Quicklime 2 tons to acre
40 ft
Plot F
70 ft
Formalin 1 % 2000 gallons to acre
Foot-path
Plot B
Control
Fence
Plot E
40 ft
Control
Plot D
Quicklime 5 tons to acre
Plot C
Quicklime 3 tons to acre

On the 2nd December Mr Bell inspected the plots and wrote "noticed that a fair number of plants had died, though in some cases evidently from drought, from inherent weakness as well as from disease."

On December 21st he counted the surviving plants and submitted the following report: "Those unmistakably diseased were counted separately from the apparently healthy plants, and as far as my judgment went a distinction being made between 'Wilt' and 'Eelworm.'" Mr Bell's results are tabulated below[1].

Report on Plots examined 21st December, 1914.

Plot	Section	Original total	Healthy plants	Wilting	Eelworm	Blanks
A.	1	120	36	1	1	82
Quicklime	2	150	132	—	—	18
2 tons to	3	90	74	1	—	15
acre	4	90	35	—	—	55
	5	210	141	6	4	59
	6	480	222	—	—	258
	Totals ...	1140	640	8	5	487
B.	1	120	22	1	4	95
Control	2	150	119	—	1	30
	3	90	73	4	—	17
	4	90	6	—	7	77
	5	210	148	4	10	48
	6	480	132	19	18	311
	Totals ...	1140	500	28	40	578
C.	1	120	47	3	10	60
3 tons	2	150	90	2	5	53
quicklime	3	90	67	4	10	9
to the acre	4	90	27	5	8	50
	5	210	111	21	31	47
	6	360	98	2	31	229
	Totals ...	1020	440	37	95	448
D.	1	120	53	2	11	54
5 tons	2	150	89	—	4	57
quicklime to	3	90	79	2	1 (1 broken)	7
the acre	4	90	43	3	8	36
	5	210	92	5	55	58
	6	360	122	16	37	185
	Totals ...	1020	478	28	116	397

---

[1] [The numbers given in the tables are not in all cases exactly correct, as the horizontal and vertical totals do not always agree with the sums of the items (Ed.).]

Plot	Section	Original total	Healthy plants	Wilting	Eelworm	Blanks
E.	1	120	15	9	22	74
Control	2	150	100	3	11	36
	3	90	72	2	2 (4 broken)	10
	4	90	36	—	7	47
	5	210	63	14	40	93
	6	330	86	7	21	216
	Totals ...	990	372	35	103	476
F.	1	120	19	4	12	85
2000 gallons	2	150	79	—	10	61
1 % formalin	3	90	85	3	—	2
to acre	4	90	19	9	19	43
	5	210	70	10	44	86
	6	330	96	12	23	199
	Totals ...	990	368	38	108	476

In the table below the results of the experiments are reduced to percentages calculated on the totals in each plot.

	Plots ...	*A*	*B*	*C*	*D*	*E*	*F*
	How treated	2 tons quicklime to acre	Control	3 tons quicklime to acre	5 tons quicklime to acre	Control	2000 gallons 1 % formalin to acre
	Original number of plants	1140	1140	1020	1020	990	990
21 Dec. 1914	% wilting	·701	2·45	3·62	2·24	3·53	3·83
	% blanks	42·71	50·701	47·84	36·96	48·08	48·08
	% healthy	56·14	43·85	43·3	46·86	37·57	37·15

These experiments would tend to indicate that (1) formalin has no beneficial result on the disease; (2) the plots treated with quicklime were slightly better than the controls but other experiments would be necessary before arriving at any definite conclusion.

D. METHODS OF CONTROL.

The fungus causing this disease is essentially a soil organism. Methods of control should hence aim at preventing soil from becoming contaminated with the fungus rather than getting rid of it when once in the soil.

Soil once contaminated undoubtedly retains the disease germs for years and the organism can only be got rid of by disinfecting the soil. Thus Sturgis suggests disinfecting the beds with steam, a method which as yet has not proved practicable on a large scale though undoubtedly excellent for greenhouse purposes in which case the whole building should be disinfected.

The most practical method would appear to resort to crop-rotation, a process which has proved itself a cleansing factor in garden economy. Even if in order to practise crop-rotation it should be necessary to have additional ground, the money will be well spent, and at the end will be found more profitable than placing cuttings in diseased ground.

When once the disease is noticed prompt action is necessary. Diseased plants should be immediately uprooted and destroyed; to leave them standing or to dig them in would simply mean propagating the disease.

It has been shown that soil organisms are also spread by: (1) water running from or through a higher diseased area to a healthy lower area; (2) through the soil adhering to the boots of gardeners or feet of animals; (3) by using tools on an infected area and, without first sterilising, transferring them for use on healthy soil.

Attempts should be made as far as practicable to prevent the disease from spreading by one or any of the above methods.

Sturgis[1] had reason for believing that the fungus may be carried over in diseased cuttings. I, too, have observed instances where this appears highly probable and would strongly suggest that growers take cuttings only from healthy plants and lay them in in a healthy soil. As far as possible, it would appear advisable for growers to grow their own stock and not resort, to the, at times, objectionable process, frequently accompanied by serious consequences, of obtaining cuttings from their neighbours.

SUMMARY.

1. The disease in the carnation here commonly referred to as "Wilt" or "Crown-rot" is caused by a fungus belonging to the genus *Fusarium*.

2. The disease usually shows as a wet rot of the stem just below the soil. The lower leaves turn a sickly colour, are usually more erect and soon the whole plant is dead.

3. This fungus was isolated from diseased carnations; grown in

---

[1] Sturgis, Wm. C., *op. cit.* p. 1.

the laboratory and when inoculated into healthy plants produced the disease.

4. The organism grew well on all culture media, producing usually a greyish growth. Along the glass of the tubes and the bottom of Petri dishes, it showed shades of grey, yellow, brown and red.

5. The fungus grows best at 25°–30° C., with something in favour of the latter temperature. No growth at 0° C. or 40° C. At 15° C. growth less vigorous than at 25° C.: at 37° C. it remains feeble. The fungus is not killed by incubation at 0° C.; cultures from 0° C. and 37° C. transferred to 30° C. showed a vigorous growth. No growth occurred in cultures transferred from 40° C. to 30° C.

6. The organism appears to be strictly aerobic.

7. It withstands high degrees of acidity but less alkalinity.

8. Conidia vary in size in different media and range between $14{\cdot}85–37{\cdot}95\mu \times 3{\cdot}3–6{\cdot}6\mu$, hyaline, fusiform to nearly straight, on some media (banana) very small; 2–6 celled.

9. Experiments were carried out in the laboratory with various disinfectants and fungicides; these experiments serve to act as a guide for experiments on the treatment of infected soil.

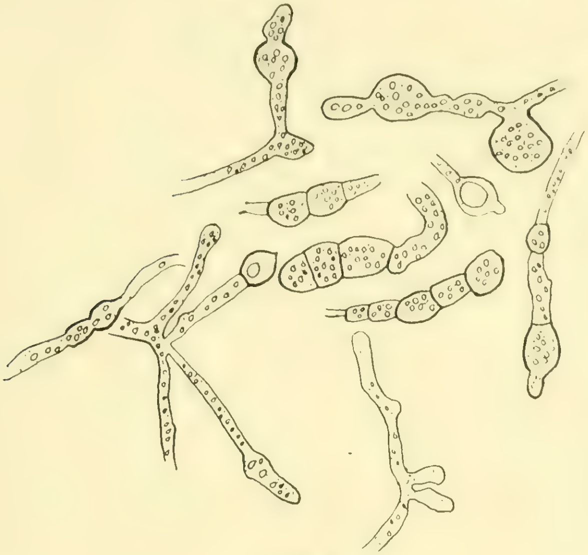
10. Field experiments carried out with the object of finding whether the application of quicklime or formalin solution to diseased soils would have any beneficial effect, tend to show: (*a*) the plot treated with formalin was no better than the control plots; (*b*) on the whole, the plots treated with quicklime fared a little better than the controls, but further experiments would be necessary.

11. It is urged that the spread of the disease should be prevented as far as practicable by: (*a*) growing one's own cuttings; (*b*) obtaining cuttings from healthy vigorous plants only; (*c*) laying cuttings in in healthy ground; (*d*) pulling up and destroying all plants showing any evidence of disease.

12. Diseased soil should be submitted to a judicious process of crop-rotation for a few years, and all attempts made to prevent the fungus spreading from diseased to healthy areas.

13. The question of the existence of varieties more or less immune from the disease has thus far not received attention, though it is a problem well worth serious consideration, and the breeding of disease-resistant varieties may yet be the ultimate solution.

THE BOTANICAL LABORATORIES OF THE
UNION OF SOUTH AFRICA, PRETORIA.
January, 1915.



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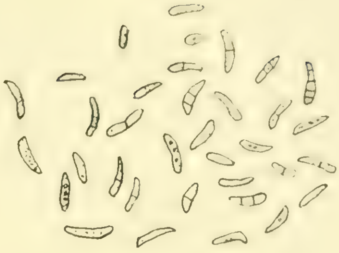

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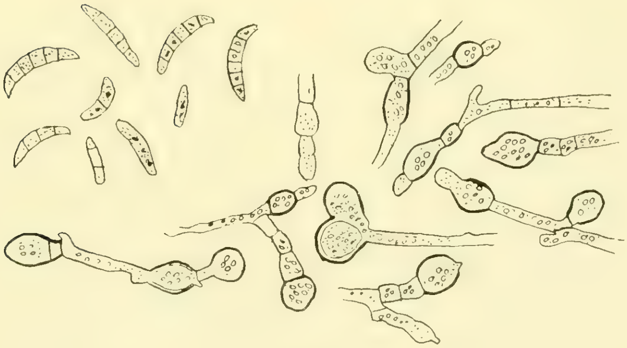

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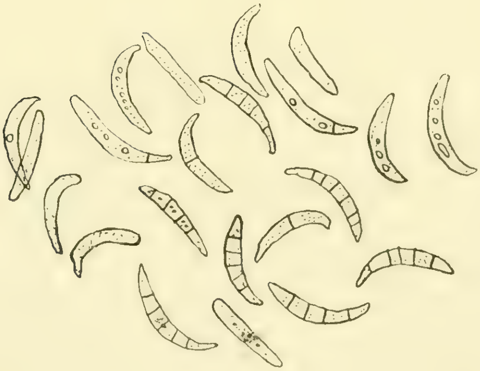

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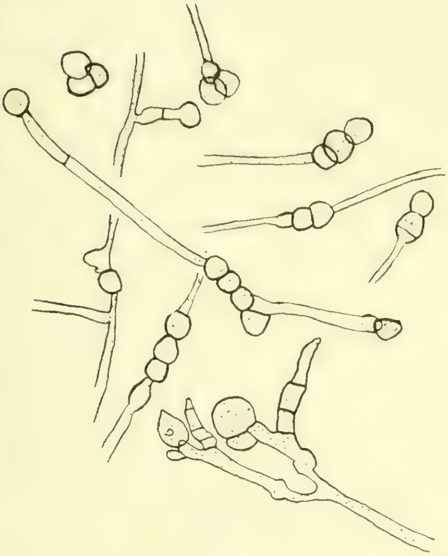

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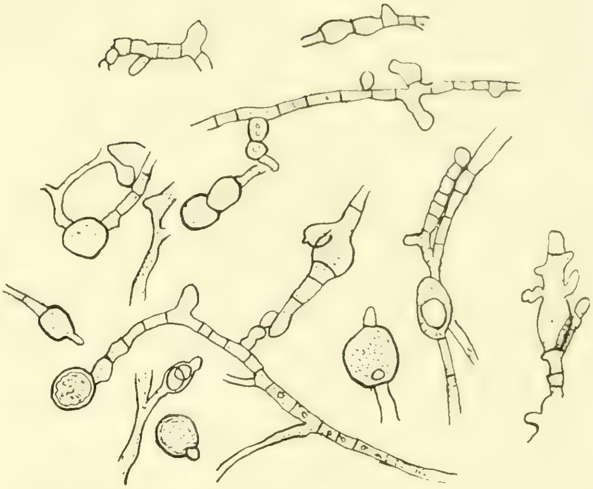

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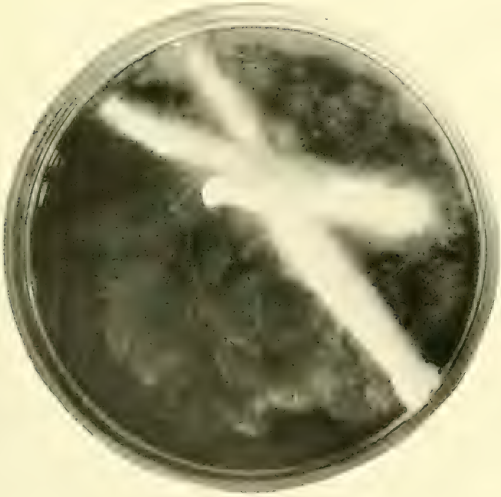

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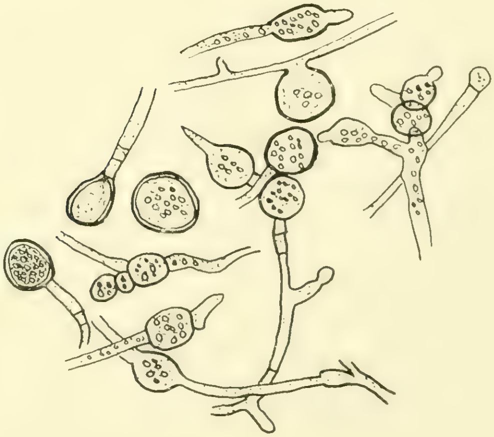

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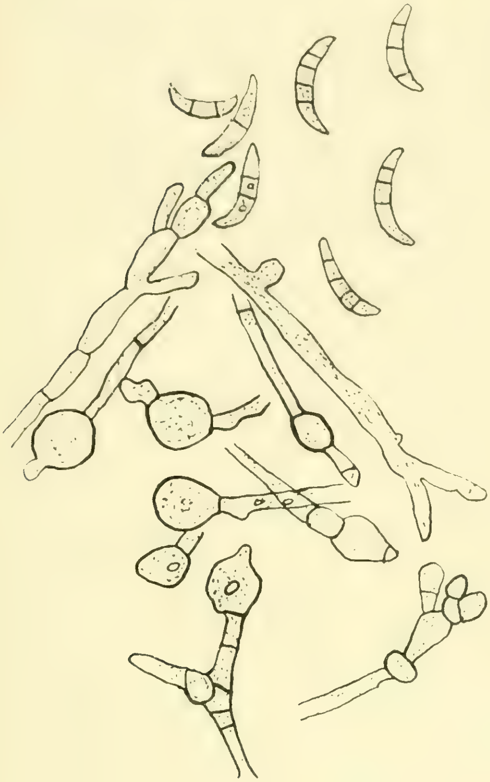

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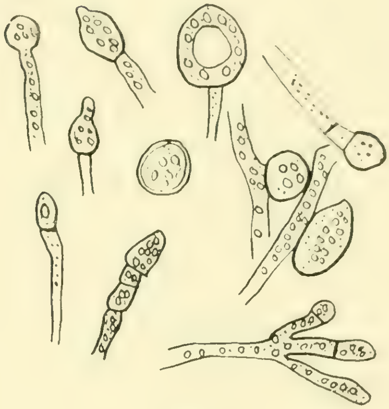
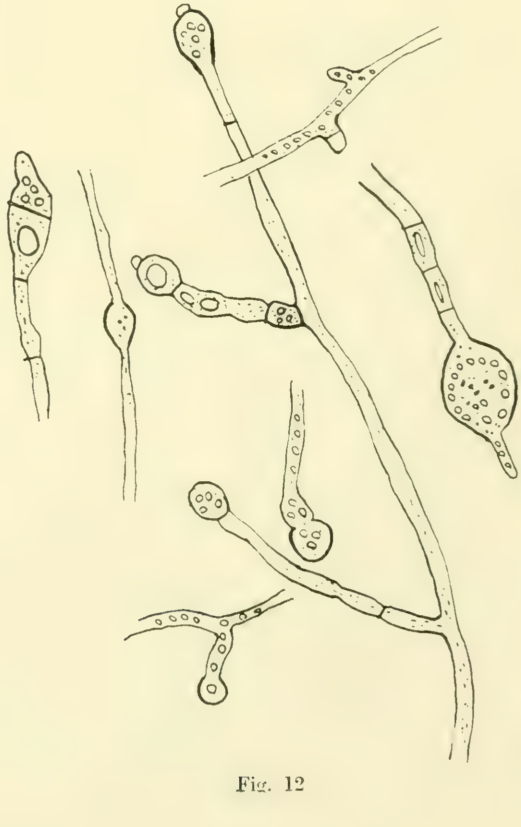


Fig. 10

Fig. 11



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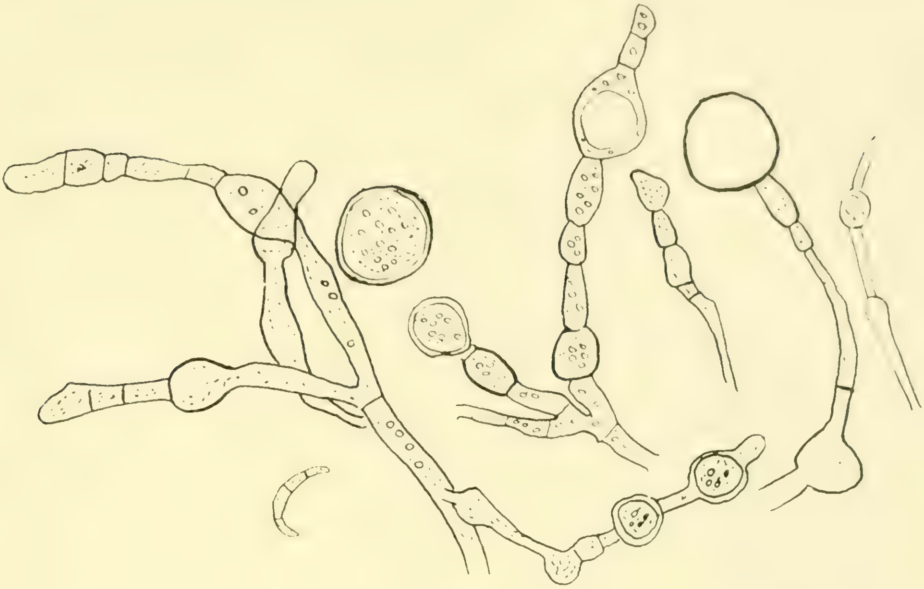

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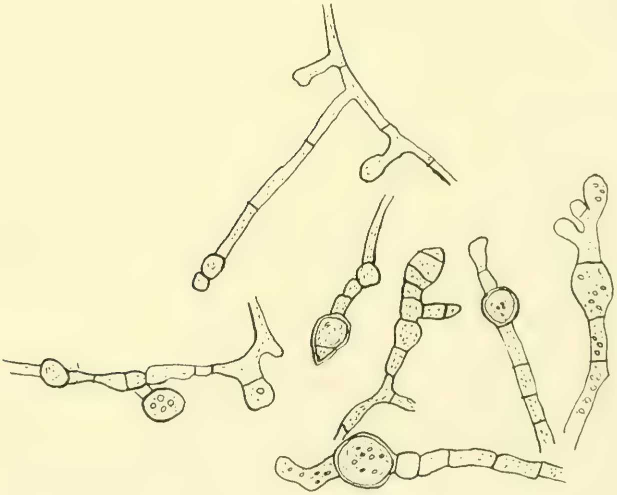

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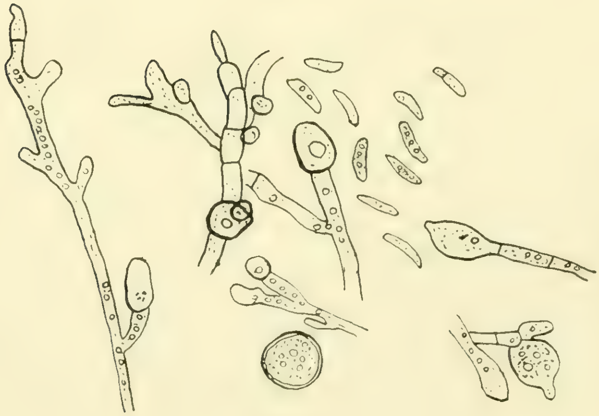

Fig. 15

EXPLANATION OF PLATES.

PLATE XXXVII.

Fig. 1 (× 400). Drawing of fungus on banana. 12 days 25° C.
Fig. 2 (× 400). Spores of the fungus on banana. 18 days 25° C.
Fig. 3 (× 400). Drawing of fungus and spores on sweet potato. 8 days 25° C.
Fig. 4 (× 400). Spores on sterilised carnation stalks. 4 days 25° C.

PLATE XXXVIII.

Fig. 5 (× 400). From sterilised carnation leaves.
Fig. 6 (× 384). From potato plug. 5 days 25° C.
Fig. 7. On sterilised carnation leaves. 5 days 25° C.
Fig. 8 (× 400). Beerwort agar, from growth growing up sides of Petri dish. 12 days 25° C.

PLATE XXXIX.

Fig. 9. Fungus and spores on potato agar. 10 days 25° C.
Fig. 10 (× 400). Fungus from liquid beerwort. 14 days 25° C.
Fig. 11. Photograph showing wart-like bodies formed in Uschinsky's solution. 14 days 25° C.
Fig. 12 (× 400). Fungus from Uschinsky's solution. 14 days 25° C.

PLATE XL.

Fig. 13 (× 400). From solution N and agar. 11 days 25° C.
Fig. 14 (× 400). From Raulin's fluid. 21 days 25° C.
Fig. 15 (× 400). From turnip tubes. 4 days 25° C.

REVIEW

Dr J. A. NELSON. *The Embryology of the Honey-Bee.* (Princeton University Press, London; Oxford University Press, 1915.) Pp. 282. Price 8*s.* 6*d.*

In this little book, although the author is "Expert in Bee-Culture Investigations" in the Department of Agriculture, Washington, we find no reference whatever to any of the problems suggested by bee-culture: we have only an account of the early stages in the development of the bee up till the time that it emerges as a grub from the egg-shell and takes up its existence within a cell of the honeycomb. To the student of comparative embryology however as distinguished from the economic zoologist the book is welcome, for it gives a most painstaking and thorough account of the formation of the blastoderm in the egg, of the differentiation of ectoderm, endoderm, and mesoderm, and of the manner in which these three layers are transformed into the most important organs of the grub. About the formation of these layers in insects there had developed a good deal of controversy. Heymons had maintained that in the higher insects, the endoderm, after giving rise to cells which assisted in liquefying the yolk, disappeared, and that the midgut was lined by ectodermal cells which grew in from the mouth and anus. Now this view, which would upset all our ideas as to the fundamental functional distinctions between the germ-layers, was opposed by Hirschler who maintained that Heymons had been misled by gaps in the series of stages which he examined, and that the endoderm in the higher insects as in the lower forms the epithelium of the midgut. In the volume before us Dr Nelson gives convincing proof that Hirschler is right at least so far as the honey-bee is concerned. The book is admirably illustrated: a number of plates illustrating whole mounts are collected at the end, whilst interspersed with the text are a number of figures illustrating the development of particular organs. The technique employed is carefully explained and there are a number of valuable hints on the effects of different kinds of preserving fluids. The book will constitute a welcome addition to the library of the scientific zoologist.

E. W. M.

457 D

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